MANAGEMENT OF PLANT-PARASITIC NEMATODES ON PEACH UTILIZING POST-PLANT NEMATICIDES AND CROP ROTATIONS

by

ANDREW MITCHELL SHIRLEY

(Under the Direction of Phillip M. Brannen)

ABSTRACT

Given the rising cost of pre-plant fumigant applications, like Telone II (1,3-dichloropropene) and Vapam (metam-sodium), the question of their future availability, and the fact that effective control of nematodes by fumigants is short lived, much effort has gone towards developing sustainable post-plant nematode control options in perennial crops, like peach. In the greenhouse, two post-plant nematicides were applied to nematode-infested peach seedlings and evaluated for their suppression of nematode reproduction. At 40 days after inoculation (DAI), a single application of Movento at (0.42 kg/ha) and MCW-2 at (0.014 kg/ha) significantly reduced *M. incognita* populations; no effect was seen at 70 DAI. At 30, 60, and 90 DAI MCW-2 at 0.014 kg/ha significantly reduced *M. xenoplax* numbers; no effect was seen with Movento at 30, 60, or 90 DAI. A dual application of Movento reduced *M. incognita* numbers at 0.42 and 0.63 kg ai/ha, 40 DAI; with no effect observed 70 DAI. A dual application of Movento on *M. xenoplax* infested plants had no effect at 30, 60, and 90 DAI. In a separate study, the host susceptibility of Jesup (Max-Q) tall fescue was evaluated against *M. floridensis* in a series of greenhouse trials. *Meloidogyne floridensis* was found to reproduce on Jesup (Max-Q), but was classified to be a poor host in two of three trials.

INDEX WORDS: Endophyte, host-parasite relationship, management, *Meloidogyne arenaria*, *Meloidogyne floridensis*, *Meloidogyne incognita*, *Mesocriconema xenoplax*, PTSL, resistance, ring nematode, root-knot nematode, *Schedonorus arundinaceus*, tall fescue grass.

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DEDICATION

This is dedicated to my entire family for being a positive support throughout my academic career and for giving me the freedom to make my own choices, regardless if they were sensible or not.

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CHAPTER 1

INTRODUCTION

In the state of Georgia, peach [*Prunus persica* (L.) Batsch] production is a $31.3 million industry (2012 USDA Georgia Agricultural Facts), with production ranking third behind South Carolina and California in the USA. Considering the importance of peach production to the state of Georgia and the Southeast, the need for better nematode management practices has become apparent. The industry is constantly dealing with new struggles which have taken away from and hindered the ultimate success of peach production in the Southeast and even the rest of the nation. Increases in labor costs and lack of competent labor have been two major concerns for many producers who struggle to find help in harvesting their crop each growing season. With the uncertainty of the effects of global climate change, increased demand for "higher quality" fruit, the threat of the introduction of exotic pests and diseases, and a demand for alternatives to chemical pesticides (e.g., fungicides, insecticides, and nematicides), there is a need for new management practices. This will require a sustainable system of management practices and IPM strategies, which includes nematode control. A portion of these challenges will be met with the goal of researching new cultural practices (in terms of planting, rotations, cover crops, etc.), reductions in chemical inputs, and alternatives to chemicals for the suppression of plant-parasitic nematodes.

Peach production in the southeastern United States dates back to the late 1600s, and reports of peach replant issues and disease in orchards are just as old (Brittain and Miller, 1978). The southeastern US, particularly Georgia, have long been known for its peach production. This

is partly due to the regions favorable climate, soil types, and market availability. Despite the success of peach production in the Southeast, peach acreage has decreased drastically over the last 10 years (2002 to 2012) from 150,000 acres to 120,000 acres. Much of this decrease is attributed to the impact of nematodes [either associated with Peach Tree Short Life (PTSL) or peach tree decline], diseases (e.g., Armillaria root rot), and environmental factors leading to a reduction in peach tree survival and productivity. In recent years, disease management and nematode control options for producers have become much more limited. Producers are dealing with the loss of methyl bromide, the increased cost of remaining fumigants [e.g., Telone II (1, 3-Dichloropropene)], and possibly the eventual loss of tolerance in current rootstocks, due to nematode diversity. There is a need for new management/cultural practices and alternative chemicals for controlling peach nematode pathogens which will provide the producer with optimum productivity. Peach producers in the Southeast are primarily concerned with three genera of plant-parasitic nematodes known to be pathogenic on peach, they include: ring (*Mesocriconema xenoplax* (Raski) Loof & de Grisse (= *C. xenoplax* (Raski) Luc and Raski), root-knot [*Meloidogyne incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. floridensis* Handoo et al.], and root-lesion (*Pratylenchus vulnus* Allen and Jensen) nematodes (Nyczepir and Esmenjaud, 2008). For these studies we will focus mainly on the ring and root-knot nematodes.

The ring nematode, *M. xenoplax* is arguably one of the most important nematode pathogens on peach due to its association with the disease complex known as Peach Tree Short Life (PTSL) (Nyczepir, 1989; Nyczepir et al. 1983; Brittain and Miller 1978). In a survey of commercial peach orchards in South Carolina and Georgia, this ring nematode was detected in 100% of soil samples collected in those orchards where PTSL was present (Nyczepir et al.,

1985). The PTSL disease complex is caused by the predisposition of young peach trees (approximately 3-5 years in age) to cold injury, bacterial canker caused by (*Pseudomonas syringae pv. syringae* van Hall*)*, or a combination of both resulting from parasitism by the ring nematode (Brittain and Miller, 1978; Nyczepir et al. 1983). Wilting of young foliage and discolored cambial tissue first show up in the peach orchard in the late winter or early spring, followed by the sudden collapse of new growth above the soil line which eventually leads to the death of all the aboveground portions of the tree; bacterial canker is the most frequently observed symptom (Nyczepir et al. 1989). Sometimes trees weakened by cold injury and/or bacterial canker may also be invaded by the fungus *Luecostoma persooni* as a secondary infection of damaged tissue (Ritchie and Clayton, 1981).

Peach tree decline, unlike PTSL, is associated with the root-knot nematode and the root-lesion nematode (*P. vulnus*) (Nyczepir, 2011; Ritchie and Clayton, 1981). The root-knot nematodes are by far the most damaging and prevalent plant-parasitic nematodes in the world and are found in all agricultural production areas ranging from temperate to tropical climates (Lamberti, 1979; Sasser, 1979; Sasser and Freckman, 1987). The root-knot nematodes, *M. incognita* and *M. javanica* were found in 95% and 5% of peach orchards surveyed in South Carolina, respectively (Nyczepir et al. 1997). A newly identified nematode *M. floridensis*, the peach root-knot nematode, previously described as *M. incognita* (Handoo et al. 2004), has been shown to parasitize resistant peach rootstocks Nemaguard and Guardian®, which are both known to be resistant to *M. incognita* and *M. javanica*. Currently the only peach rootstocks with known resistance to *M. floridensis* are Flordaguard, MP29, and Sharpe (Beckman et al. 2012; Beckman et al. 2008; Nyczepir et al. 2006). Currently this nematode is only found in Florida, though *M. floridensis* could pose a major economic issue to growers throughout the Southeast. The

distribution of this nematode is currently limited to seven continuous counties within Florida. Given this, *M. floridensis* could easily be disseminated to other peach growing regions throughout the Southeast (Brito et al 2008; Brito et al. 2010).

As a plant-parasitic nematode on peach, the root-knot nematode's aboveground symptoms include; a reduced fruit yield, decreased plant vigor, and promotion of early defoliation in severely stunted plants. Belowground symptoms include reduced root systems with malformation and galling present. Under severe conditions, these symptoms can even lead to tree death (Nyczepir et al. 1993). Since the root-knot nematode is an obligate sedentary endoparasitic nematode, the use of post-plant nematicides should offer measurable control.

Currently the only pre-plant fumigant chemicals available to peach producers in the Southeast are Telone II and Vapam, with Telone II being the one primarily being used and recommended to growers (Horton et al., 2013). Methyl bromide (bromomethane) was once a recommended preplant nematicide option for peach growers, but its importation and manufacture has been banned in the USA since January 2005, due to its ozone depleting properties (Clean Air Act, 1990). Along with methyl bromide, producers have lost the nonfumigant fenamiphos, the only remaining post-plant nematicide recommended for use on peaches in the Southeast. The manufacturers of fenamiphos canceled all product registrations of the chemical, due to its human health risks and the costs associated with its re-registration in May of 2007. Due to a lack of pre and post-plant nematicidal options afforded to peach producers and the increased cost of those that remain, reduced rates of soil fumigants, alternatives to chemical controls, nematode non-host groundcovers/rotation crops, biorational nematicides, and improvements to our cultural practices are becoming ever more important.

Currently, soil fumigation with Telone II is the preferred control method for most plant-parasitic nematodes in peach. Use of Telone II, however, requires high application rates which can be toxic to mammals and poses an environmental risk. The recommended broadcast rates for peach in the Southeast are 250-327 liters/ha (27-35 gal/acre) (Horton et al., 2013). The recommended rate for strip-application in peach is 28 liters/ha (3gal/acre); a 10-fold difference in Telone II usage in strip-application. Though both broadcast and strip-applications are recommended for nematode control in peach, most producers tend to go with a strip-application, due to cost-effectiveness; growers can save 40% on application costs vs. broadcast application (Browne et al. 2007). In these studies the use of even lower rates of Telone II will be evaluated for efficacy in nematode control through strip-application in combination with resistant Guardian rootstock.

Historically, peach production and IPM strategies for nematodes have relied almost solely on pre-plant applications of soil fumigants (Nyczepir, 1989). There has recently been an interest in the development and use of more environmental-friendly post-plant nematicides as alternatives to soil fumigants. There are currently three nonfumigant chemicals being tested which have demonstrated nematicidal activity. These include, Movento (spirotetramat, a synthetic tetramic acid, Bayer Crop Science currently marketed as a broad-spectrum insecticide), MCW-2 (fluensulfone, Makhteshim-Agan Industries), and GA534 (extracted mycotoxin).

Spirotetramat is naturally derived from fungi and other organisms. The compound has a very low level of mammalian toxicity (>5000 mg a.i./kg bw) (Movento website, BayerCropScience). It is transported through both the phloem and xylem (ambimobile); once inside the leaf it is hydrolyzed to its –enol chemical form, and is then moved through the phloem and xylem to both leaf and root apical meristems. It is a Group 23 lipid biosynthesis inhibitor

that acts on fecundity (number of eggs) and fertility (viable eggs) when ingested by the target organism. It has also been observed to affect edysis in aphids, leading to the incomplete shedding of the cuticle during molting. Soil activity is very short-lived with approximately 90% dissipation in one to four days; however, it has residual activity in planta for two or more weeks (Bruck et al. 2009; Smiley et al. 2011, 2012; McKenry et al. 2009, 2010; Zasada et al. 2012). McKenry et al. (2009) applied spirotetramat at <100 ml/ha to *Vitis*, *Citrus*, and *Juglans* spp. and observed a reduction in populations of *Xiphinema* spp and *M. xenoplax* at 36 and 56 days after treatment, respectively. If irrigation was withheld for up to two weeks, a 50% population reduction was observed for three months for all plant-parasitic nematodes, to include *Meloidogyne* species. In a separate study, the effect of spirotetramat on *P. vulnus* populations in *Juglans* spp. roots was evaluated for six months. A 50% *P. vulnus* population reduction was observed when applied at a rate of 441ml/ha with an adjuvant (McKenry et al. 2010). Smiley et al. (2011) applied spirotetramat at 88 g/ha to two wheat fields, one in Idaho and the other in Washington, infested with the cyst nematode, *Heterodera avenae*. Results indicate that spirotetramat reduced *H. avenae* population densities by 35% and 78% in the Washington and Idaho field trials, respectively. Movento may be a promising post-plant nematicide for the control of plant-parasitic nematodes on peach.

MCW-2, fluensulfone, a new product from Makhteshim-Agan, has also been shown to be a promising post-plant nematicide. Fluensulfone belongs to the fluoroalkenyl group, and it kills nematodes on contact. Fluensulfone has good soil residual activity, a new mode of action, is root systemicity, and has no insecticidal effects. The compound also has low mammalian toxicity (500-1000mg/kg), making it less toxic than aldicarb, fenamiphos, and oxamyl, while being non-toxic to honey bees and birds (Everich and Schiller, 2009). Fluensulfone is generally applied by

drip irrigation or through a drench. Recent studies by Oka et al. (2009) evaluated fluensulfone for control of *M. javanica* on tomato. Fluensulfone was applied at rates of 0.5, 1, 2, and 4 mg/L as a drench and compared with fenamiphos and cadusafos. At all the rates, MCW-2 significantly reduced numbers of root galls and eggs as compared to the control treatment (Oka et al. 2009). Zasada et al. (2010) conducted a similar trial comparing MCW-2 along with other post-plant nematicides against *P. penetrans* on raspberry. It was observed that a drench application rate of 9.9 kg ai/ha reduced the total number of root-lesion nematodes recovered from the soil compared to the control in one trial, but not in a second trial. In another study MCW-2 was applied at 2.1, 4.2, 6.3, 8.3 L/ha pre-plant and 8.3 + 4.2 L/ha pre-plant/ post-plant. All of the treatments, except 4.2 L/ha, had a significantly lower gall rating compared to the control (Driver and Louws, 2010). MCW-2, like Movento, has good potential as a post-plant nematicidal option in the peach producer's arsenal. This product may potentially serve as a replacement, if not a better control method, for the previous post-plant nematicide fenamiphos.

GA534 is a biologically derived nematicide developed and evaluated for the control of root-knot nematode, *M. incognita*, in cotton. The product is a fungal culture filtrate obtained from the GA534 isolate (species is confidential), and has been shown to significantly suppress root-knot nematode reproduction 120 days after planting when applied as a soil drench at the base of growing cotton plants. Evaluation of this product was conducted at four different cotton field sites in Georgia in 2009. Results indicated that there was approximately a 55% reduction in *M. incognita* J2 and egg population in plots treated with GA534 (Noe, 2009). Like the two other bio-rational nematicides previously mentioned, GA534 may provide an improved post-plant control strategy for peach nematode pathogens in the Southeast. This product is naturally derived

and environmentally-friendly; if given proper certification, it could also provide organic producers with a useful nematicide.

The use of pre-plant rotation with groundcovers could serve as a management practice to reduce plant-parasitic nematode populations and any associated disease. In the Southeast when a peach orchard is removed due to severe stunting from root-knot nematode damage or PTSL from ring nematode, the currently recommended practice is to apply pre-plant fumigation using Telone II along with a resistant rootstock to insure increased tree longevity and maximum nematode protection (Horton et al. 2013). Peach growers often find it difficult to afford the costs associated with pre-plant fumigation and/or are unable to apply the fumigants at the proper time of the year due to management conflicts with other crops. These issues have led to a growing interest in the use of suppressive groundcovers as a nematode management strategy in peach producing areas. One groundcover that has shown to be a promising option is the tall fescue grass cultivar Jesup (Max-Q); Max-Q is a non-toxic endophyte (*Neotyphodium coenophialum*) infested tall fescue developed as a viable forage crop for cattle production in eastern USA and some areas in the West. Its growing popularity among producers is due to the presence of a novel fungal endophyte that does not produce ergot alkaloids that cause fescue toxicosis, but does impart drought tolerance (Phillips et al., 2009). Max-Q has been shown as a non-host/poor host to a number of nematodes. In a recent study the host status of Max-Q was tested against four *Meloidogyne* spp. It was determined that Max-Q is a non-host to *M. incognita* and *M. hapla*, a poor host for *M. javanica* and a good host for *M. arenaria* (Nyczepir and Meyer, 2010). Also the host status of Max-Q was determined for *M. xenoplax* and *P. vulnus*, it was shown that Max-Q is a poor host to *P. vulnus*, but a good host to *M. xenoplax* (Nyzcepir, 2011). The host status of Max-Q to a newly described root-knot species on peach, *M. floridensis*, and the length of time

needed for rotation of this crop prior to planting the orchard site back to peach is not known. Given the host status of Max-Q to the nematodes mentioned above, this plant can potentially function as a good candidate for a preplant groundcover rotation strategy in suppressing those nematodes which do not survive or that poorly reproduce on Max-Q fescue.

Research Objectives and Goals

1. Evaluate the efficacy of biorational nematicides for controlling ring and (or) root-knot nematode in peach

   Goal: Provide the peach industry with new post-plant nematicides for controlling ring and (or) root-knot nematodes in peach. Post-plant control is absolutely essential to extend the life of peach trees on PTSL sites, since the nematode populations increase and re-establish in the years subsequent to pre-plant fumigation.

2. Develop improved nematode management strategies based on cultural approaches for suppression of ring and (or) root-knot nematode and related peach disease complexes

   Goal: Provide the peach industry with a new groundcover which suppresses nematode population densities comparable to pre-plant fumigation

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CHAPTER 2

LITERATURE REVIEW

Peach Production and Nematode Impact

The economic value of peach production in the USA is estimated at around $600 million, with production increasing in value by $100 million in the past 10 years. In 2011, the state of Georgia valued peach production at $31.5 million. Though the value of peach production has increased in the past 10 years in the USA, the amount of bearing hectares planted has decreased from 59,000 hectares (145,000 acres) planted in 2002 to less than 46,000 hectares (115,000 acres) planted in 2012. In the state of Georgia the bearing hectares has decreased from 4,400 hectares (10,900 acres) in 2002 to less than 4,000 hectares (10,000 acres) in 2012 (USDA Agricultural Statistics 2012). Much of this decrease is due to disease development within the orchard, including nematode associated diseases like Peach Tree Short Life (PTSL) and peach tree decline.

Nematode losses in peach can often be overlooked and go undiagnosed for many years. Nematode damage is generally variable within an orchard. Often tree losses are gradual and increase over time with a rise in nematode population (Nyczepir, 2011). Many times this trend will continue until a large concentration of trees are lost or production is greatly reduced within a given area. In Georgia, nematode losses are rather sporadic and lower than other states. Most growers in Georgia plant new orchards on sites with no previous history of peach to avoid having to use preplant fumigation, due to the high costs and to avoid any soil-borne pathogens that may have built-up over time (e.g., *M. xenoplax*) (Nyczepir et al., 2004). Commonly the producer is

not aware of potential losses from nematodes, and puts no resources into nematode control. When losses do occur they are generally quite severe with a total loss in some areas. In South Carolina, most of the nematode-associated tree losses are due to PTSL, resulting in an average loss of 143,000 trees and $11 million of income each year (Bertrand, 1994). Of this total loss it is estimated that around $6 million is lost to PTSL each year (Miller, 1994). Peach orchards have been known to survive as long as 25 to 30 years in parts of the Southeast, though many peach trees are lost to PTSL in the first 3-5 years of planting. A successful orchard is one which will survive longer than 10 years before having to be removed (Ritchie and Clayton, 1981).

Peach Tree Short Life (PTSL)

For more than 300 years peach producers have been dealing with peach replant issues. Most of the losses observed were found in old peach sites where newer orchards were established. These issues however are not restricted only to old sites, but can occur in newly planted sites with no history of peach production (Brittain and Miller, 1978). One of the issues of major concern is the disease complex Peach Tree Short Life (PTSL). The PTSL complex is caused by the susceptibility of young peach trees (approximately 3-5 years in age) to cold injury and/or bacterial canker caused by (*Pseudomonas syringae pv. syringae* van Hall). Symptoms show up in the peach orchard in the late winter or early spring as the sudden collapse of new growth above the soil line, eventually leading to the death of all the aboveground portions of the tree, with bacterial cankers being the most frequently seen symptom (Nyczepir et al. 1989). A dying or weakened tree with symptoms of cold injury and/or bacterial canker may also be secondarily invaded by the fungus Cytospora, *Luecostoma persooni*, through the cold-damaged or diseased bark (Ritchie and Clayton, 1981).

Symptomatology

Symptoms of this disease complex occur on peach trees which typically appear as healthy productive trees the year before. One common symptom is the unexpected collapse of growth and eventual death of a young tree (Brittain and Miller, 1978; Beckman and Nyczepir, 2011; Nyczepir, 1989; Ritchie and Clayton, 1981), which are similar to the symptoms observed on any plant deprived of water caused. Removal of bark in the affected areas will show a transition between healthy and brown necrotic vascular tissue. At this division the characteristic brown tissue will extend and follow down the tree trunk to the soil line, where it ceases. This brown tissue will give off a distinct "sour sap" smell which is characteristic of PTSL. This odor is caused by the fermentation of carbohydrates released by the plant's damaged cambial tissue. This symptom may also occur along with water-soaking of the bark and leakage or ooze from lenticels within the bark. The primary root system below the soil line remains alive and appears healthy, but upon further examination of the feeder roots one will detect unhealthy, necrotic roots and a reduced number of tertiary roots caused by parasitism from *M. xenoplax*. In mid- to late summer, suckers may form at the base of the trunk from the surviving root system (Brittian and Miller, 1978; Nyczepir, 1989). Symptoms associated with bacterial canker usually coincide with a delay in flowering and leafing out in the spring, with decline and death of the affected limbs or tree by late summer. Cracking of damaged bark may occur, and it can easily be peeled away from the limbs and trunk of the tree (Beckman and Nyczepir, 2011).

Disease development

The warning signs of PTSL are present if one knows when to look for them. Early symptoms include; dead feeder roots, yellowing and premature defoliation, and lack of a reaction to fertilizer applications. These symptoms begin with cold damage and subsequent bacterial canker development in the late fall or early winter. Both of these are initiated by parasitism from

the ring nematode, *M. xenoplax* (Beckman and Nyczepir, 2011; Brittian and Miller, 1978; Nyczepir, 1989; Ritchie and Clayton, 1981).

Cold damage affects healthy cambial tissue just below the bark of the peach tree. The creation of new xylem vessels is halted by cold injury to the vascular cambial layer, and when new growth emerges, the sudden uptake of water makes the tree water deficient, resulting in a sudden collapse of growth and then death. During the winter this cambial layer is highly resistant to cold injury, but it becomes more susceptible once the growth and production of new tissue has resumed. So, when this new growth is initiated the tree is no longer in dormancy. A healthy tree produces new xylem elements each year, with the old xylem becoming nonfunctional and filled with gum. After this the plant lays down a layer of new xylem tissue for the following year of growth. It is during this time period that cold damage and bacterial canker are most likely to occur (Beckman and Nyczepir, 2011; Brittian and Miller, 1978).

Bacterial canker, caused by *Pseudomonas syringae pv. syringae* van Hall, will kill dormant peach buds, limbs, branches, and the entire tree. Cold damage is very similar to bacterial canker in symptoms and must be identified early to distinguish between the two. Like cold damage, bacterial canker affects only the above-ground portions of the tree. The bark is reddish brown and cankers are elongated with a distinct margin of healthy and necrotic tissue. Data indicates that peach trees need to be predisposed by *M. xenoplax*, pruning, and other factors to become more susceptible to infection by *P. syringae* (Ritchie and Clayton, 1981). The most noticeable symptom concerning bacterial canker is the impediment or failure of individual limbs or the entire tree to successfully bloom and leaf out in early spring. In warm weather the bacterial cankers will begin to become gummy and appear sunken as compared to healthy tissue.

Generally death of the infected branches and tree follows shortly after symptom development (Brittian and Miller, 1978).

The factors that prompt PTSL disease includes, rapid changes in winter and spring temperatures, pruning in the late fall and winter (October through January), *M. xenoplax*, susceptible rootstocks, acidic soils, and any root injury. In most cases more than one of these factors are involved, but they are not all required for disease establishment. By managing these issues, the likelyhood of PTSL appearing in an orchard will dramatically decrease (Brittian and Miller, 1978; Ritchie and Clayton, 1981; Beckman and Nyczepir, 2011).

Management of PTSL

Management of PTSL should begin with site preparation. The greatest losses due to PTSL are often found in plantings located in old peach sites where the soil nutrient levels are depleted, soil pH is low, ring nematode populations have increased , and the presence of *P. syringae pv. syringae* will lead to the development of PTSL. Ring nematode presence appears to be the most important predisposing factor to consider (Nyczepir, 1990). Soil samples should be taken for nematodes, nutrient levels, and pH. Lime should be applied to bring the pH levels between 6.0-6.5 and nutrients should be applied during liming. Subsoiling the land to remove any hardpan which may exist must be done during this process. Subsoiling the new site will improve water infiltration and drainage, encourage root growth and development, increase tree survival during weather extremes, and enhance nutrient uptake (Beckman and Nyczepir, 2011; Brittian and Miller, 1978).

After soil amendments have been completed, a pre-plant application of Telone II should be administered to the soil during moderate temperatures when soil moisture is present (Horton et al., 2013). Fumigation should be applied to soils which are suitable for cultivation during the

fall, and it works best in sandy or sandy loam soil, whereas its efficacy is reduced in heavy clay or clay loam soils. This lack of efficacy is due to smaller texture size and pore space and also a tendency to retain water. Compaction may also pose a problem when applying a pre-plant fumigant, preventing the movement of fumigant throughout the soil profile, hence the importance of subsoiling beforehand. Any old plant roots should be removed prior to fumigation, to rid the soil of any possible nematodes harbored within old root material. By typing up the fumigant within the soil, areas of high organic material also can play a role in fumigation efficacy. Soil fumigants can be broadcast or strip-applied in the orchards. Strip-applications of fumigants are the most common and cost-effective in peach (Brittian and Miller, 1978; Nyczepir, 2011).

A reliable rootstock should be chosen based on site history and nematode samples. There are currently three rootstocks recommended for use in Georgia orchards, these include; Lovell, Halford, and Guardian®. Lovell has no resistance when it comes to nematodes which parasitize peach, but has some tolerance to PTSL as compared to Nemaguard. Nemaguard has resistance to the two common root-knot nematode species, *M. incognita* and *M. javanica*. This rootstock works well when root-knot is the only nematode present. Nemaguard however, is more prone to *M. xenoplax* -induced PTSL tree death than the other rootstocks mentioned above. Guardian also has resistance to both common species of root-knot, and is generally more resistant to PTSL than either Nemaguard or Lovell. Trees grown on Lovell live longer and are more productive than trees on Nemaguard in a PTSL site (Nyczepir, 2011). A newly described root-knot nematode, *M. floridensis*, the peach root-knot nematode, previously thought to be a population of *M. incognita* (Handoo et al. 2004), has been shown to parasitize resistant peach rootstocks Nemaguard and Guardian. The only peach rootstocks with resistance to *M. floridensis* are Flordaguard, MP29,

and Sharpe (Beckman e al. 2012; Beckman et al. 2008; Nyczepir et al. 2006). None of these rootstocks provide full control of nematode species and should not be substituted for pre-plant fumigation.

Another management strategy component which may be considered for control of nematodes is crop rotation. A producer may plant a crop which doesn't allow for the feeding or reproduction of the nematode and a rotation crop may work best for control of root-knot nematode on peach. Another advantage to a rotation scheme would be the benefit of some added income from the crop based on its harvested value. Some downsides of this method are the delay of orchard planting by an estimated two to four years and the fact that some grass crops (e.g., coastal bermudagrass and bahiagrass) which eliminate root-knot may allow for the reproduction of the root-lesion nematode, *P. vulnus*, which parasitizes peach. The rotation of land with wheat for a period of three years before planting a new peach orchard has been shown to be as successful as a pre-plant fumigation with methyl bromide in suppressing ring nematode and increasing tree survival in an old peach site with a history of PTSL (Nyczepir, 2011). It should be noted that the use of pre-plant fumigation, resistant rootstocks, and rotation will not totally eliminate nematodes and over time the population will build back up to damaging levels if no postplant management practice is utilized.

Pruning at the proper time will have an effect on the development of PTSL within an orchard. For the same reason parasitism by *M. xenoplax* causes the peach tree to be more susceptible to cold injury, pruning at the wrong time of the year can initiate premature root growth during the winter months. Damage by both interferes with the dormancy of peach (Brittian and Miller, 1978; Beckman and Nyczepir, 2011; Ritchie and Clayton, 1981). Pruning in the months from October through January has been associated with the death of trees the

subsequent spring. This practice predisposes the trees to bacterial canker, caused by *P. syringae*, and (or) cold injury. The younger trees are generally more susceptible to this damage than the older plantings. Heavy pruning is discouraged and only light pruning where needed is recommended, in order to promote and maintain a healthy root system (Brittian and Miller, 1978; Beckman and Nyczepir, 2011; Ritchie and Clayton, 1981). The proper time to prune is in the spring from February to June (Horton et al. 2013).

In conclusion, management of PTSL currently includes; proper site management, correct cultural practices, proper rootstock selection, use of a viable cover crops, along with the application of a soil fumigant. In order to better manage the disease, we need to evaluate new cover crops and potential post-plant nematicides for their control of plant-parasitic nematodes in peach.

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CHAPTER 3

USE OF SPIROTETRAMAT AND FLUENSULFONE IN THE POST-PLANT MANAGEMENT OF *MESOCRICONEMA XENOPLAX* AND *MELOIDOGYNE INCOGNITA* ON PEACH

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Abstract: Greenhouse experiments were designed to compare the efficacy of two post-plant nematicides which have shown promise in controlling plant-parasitic nematodes on peach; they include spirotetramat (Movento) and fluensulfone (MCW-2). Both chemicals were evaluated in laboratory bioassays and under greenhouse conditions for efficacy against the root-knot nematode, *Meloidogyne incognita*, and the ring nematode, *Mesocriconema xenoplax* from 2011-2013. Each chemical was applied at varying rates in separate studies for each nematode species. For the root-knot nematode studies, 'Lovell' peach seedlings were inoculated with 20,000 eggs and treatments applied 10 days later. Soil samples were collected ~ 40 and 70 days after inoculation (DAI). At 40 DAI, Movento at (0.42 kg/ha) and MCW-2 (0.014 kg/ha) significantly reduced *M. incognita* numbers ($P < 0.05$) compared to the controls; no effect was seen at 70 DAI. For the ring nematode studies, 'Nemaguard' peach rootstock seedlings were inoculated with 1,000 *M. xenoplax* and treatments were applied 10 days later. Treatments were the same as for the root-knot study, except both chemicals were included in the same study. Soil samples were collected ~30, 60, and 90 DAI. At 30 DAI MCW-2 at 0.014 kg/ha significantly reduced *M. xenoplax* numbers ($P < 0.05$) compared to the controls; no effect was seen for Movento at 30, 60, or 90 DAI. MCW-2 was also efficacious 60 and 90 DAI. Two separate studies which included a dual application of Movento were also conducted with both the root-knot and ring nematodes. Protocols were similar for both studies except a second application of Movento was applied 40 DAI. For the root-knot studies at 40 DAI, Movento reduced *M. incognita* numbers ($P < 0.05$) at 0.42 and 0.63 kg ai/ha; with no effect observed 70 DAI. For the ring nematode studies no effect was seen for Movento at 30, 60, or 90 DAI.

Key words: management, *Meloidogyne incognita*, *Mesocriconema xenoplax*, PTSL, ring nematode, root-knot nematode.

In the state of Georgia peach production is a $31.3 million industry (2012 USDA Georgia Agricultural Facts), with production ranking third behind South Carolina and California, respectively. Considering the importance of peach production to the state of Georgia and the Southeast, the need for better nematode management practices has become apparent. Given the rising cost of pre-plant fumigant applications, like Telone II (1,3-Dichloropropene) and Vapam (metam-sodium), the question of their future availability, and the fact that effective control of nematodes by fumigants is short lived, much effort has gone towards developing sustainable post-plant nematode control options in perennial crops, like peach (McKenry et al. 2009, 2010, 2011). At this time the recommendation for nematode management in peach is a pre-plant application of Telone II and the use of an appropriate resistant rootstock, if available (Nyczepir, 1991; Beckman and Nyczepir, 2011; Nyczepir, 2011; Brittian and Miller, 1978; Ritchie and Clayton, 1981). Pre-plant crop rotation treatments have also been shown to be effective and are recommended against some peach nematode pathogens in the Southeast (Nyczepir, 2000; Nyczepir and Meyer, 2010; Nyczepir, 2011; Meyer et al., 2013). While these practices are initially successful in suppressing nematode populations, a healthy orchard should last more than ten years, but after the first 2 or 3 years the nematode populations build back to damaging levels. This can threaten the productivity and life of an orchard, making it susceptible to secondary disorders like Peach Tree Short Life (PTSL), peach tree decline, and nepoviruses (Beckman and Nyczepir, 2011; Nyczepir, 2011; Brittian and Miller, 1978; Ritchie and Clayton, 1981). In South Carolina, approximately 143,000 trees are lost to disease, which equates to a loss of $11 million of income each year for the producer. Of this amount it is estimated that $6 million is lost per year to PTSL alone (Miller, 1994).

The ring nematode, *Mesocriconema xenoplax* (Raski) Loof & de Grisse (= *C. xenoplax* (Raski) Luc and Raski) is arguably one of the most important nematode pathogens on peach [*Prunus persica* (L.) Batch] due to its association with the disease complex known as Peach Tree Short Life (PTSL) (Nyczepir, 1989; Nyczepir et al. 1983; Brittain and Miller 1978). In a survey of commercial peach orchards in South Carolina and Georgia, this ring nematode was detected in 100% of soil samples collected in those orchards where PTSL was present (Nyczepir et al. 1985).

Peach tree decline, unlike PTSL, is often associated with the root-knot nematode and the root-lesion nematode (*Pratylenchus vulnus*) (Nyczepir, 2011; Ritchie and Clayton, 1981). The root-knot nematodes, *M. incognita* and *M. javanica* were found in 95% and 5% of peach orchards surveyed in South Carolina (Nyczepir et al. 1997). Aboveground symptoms associated with root-knot nematode feeding include a reduction in fruit yield, plant growth, and promotion of early defoliation in severely stunted plants. Belowground symptoms include reduced root systems with malformation and galling present. Under severe conditions these symptoms can even lead to tree death (Nyczepir et al. 1993).

Movento (spirotetramat, a synthetic tetramic acid, Bayer Crop Science) is marketed as a broad spectrum systemic insecticide. It is also labeled as a nematicide, in California only, for the control of nematodes in stone fruit and tree nuts. The compound has a very low level of mammalian toxicity (>5000 mg a.i. /kg bw) (Movento label, BayerCropScience). It is transported through both the phloem and xylem (ambimobile) and once inside the leaf it is hydrolyzed to its –enol form, and is then moved through the phloem and xylem to both leaf and root apical meristems. It is a Group 23 lipid biosynthesis inhibitor that acts on fecundity (number of eggs) and fertility (viable eggs) when ingested by the organism. It has also been observed in aphids to

affect edysis, leading to the incomplete shedding of the cuticle during molting. Soil activity is very short-lived with around 90% dispersal in one to four days; it is however residually active within the plant for two or more weeks (Bruck et al. 2009; Smiley et al. 2011, 2012; McKenry et al. 2009, 2010; Zasada et al. 2012). McKenry et al. (2009) applied spirotetramat at <100ml/ha to *Vitis* spp., *Citrus* spp., and *Juglans* spp. and observed a reduction in populations of *Xiphinema* spp. *M. xenoplax* at 36 and 56 days after treatment, respectively. A 50% population reduction was observed after three months for all plant-parasitic nematodes sampled if irrigation was withheld for up to two weeks. Sampling involving *Meloidogyne* spp., included with other species, also showed a 50% population reduction for three months (McKenry et al. 2009). Also in a separate study, the effect of spirotetramat on *P. vulnus* infected *Juglans* spp. was evaluated for six months and a 50% population reduction was observed when applied at 0.106 kg ai/ha with an adjuvant (McKenry et al. 2010). Smiley et al. (2011) applied spirotetramat at 0.088 kg ai/ha to two wheat fields, one in Idaho and the other in Washington, infested with the cyst nematode, *Heterodera avenae*, and found spirotetramat reduced *H. avenae* population densities by 35% in the Washington field and 78% in the Idaho field .

MCW-2, fluensulfone, a new product from Makhteshim-Agan, has also been shown to be a promising post-plant nematicide. Fluensulfone belongs to the fluoroalkenyl group and exhibits nematicidal activity, killing the nematode upon contact with the chemical, making it in the true sense a nematicide. Fluensulfone, a new mode-of-action chemistry, has been shown to have good soil residual activity, is partially root systemic, and has no insecticidal effects. The compound has low mammalian toxicity (between 500-1000mg/kg), making it less toxic than aldicarb, fenamiphos, and oxamyl, while being non-toxic to honey bees and birds (Everich and Schiller, 2009). Fluensulfone is generally applied by drip system or through a drench. Recent studies by

Oka et al. (2009) were conducted to evaluate fluensulfone against *M. javanica* on tomato. Fluensulfone along with fenamiphos and cadusafos, as a comparison, were applied at rates of 0.5, 1, 2, and 4 mg/L as a drench. They found fluensulfone significantly reduced galling and eggs counts compared to the control at all rates. Zasada et al. (2010) conducted a similar trial where she compared fluensulfone along with other post-plant nematicides against *P. penetrans* on raspberry. In this study, a drench application rate of fluensulfone at 9.9 kg/ha was able to reduce the total number of nematodes recovered compared to the control in one, but not in both trials. In a study out of North Carolina fluensulfone was applied at 2.1, 4.2, 6.3, 8.3 L/ha pre-plant and 8.3 + 4.2 pre-plant/ post-plant. All of the treatments, except for 4.2 L/ha, had significantly lower gall rating compared to the control (Driver and Louws, 2010). Fluensulfone like spirotetramat stands to become another potential post-plant nematicidal option for peach production. The objective of this research was to evaluate the efficacy of spirotetramat and fluensulfone against *M. incognita* and *M. xenoplax* on peach and to evaluate any plant growth differences.

MATERIALS AND METHODS

Nematode source and inoculum: The populations of *M. incognita* and *M. xenoplax* were originally isolated from peach in Georgia, and maintained on eggplant (*Solanum melongena* cv. 'Black Beauty') and peach (*Prunus persica* cv. 'Nemaguard') in the greenhouse at the University of Georgia, Athens, respectively. *Mesocriconema xenoplax* were extracted from the culture medium using the centrifugal-flotation technique (Jenkins, 1964). Eggplant roots were processed for *M. incognita* egg inoculum using a 0.5% NaOCl solution (Hussey and Barker, 1973).

Spirotetramat (Movento) and fluensulfone (MCW-2) were evaluated in greenhouse studies from 2011-2013 with the root-knot nematode, *M. incognita*, and the ring nematode, *M.*

xenoplax. Each chemical was applied at varying rates in separate studies for each nematode species.

Root-knot nematode spirotetramat and fluensulfone studies: 'Lovell' peach seedlings were transplanted into 20-cm-diameter standard clay pots containing 3.4 L sterilized loamy sand soil mixture of 25% field soil, 50% sand, and 25% Fafard® germinating mix and placed on benches in a greenhouse (~ 27 ± 5° C). After transplanting a ~1 ml (1/4 teaspoon) of 13-13-13 was applied to each pot and water was applied as needed. Plants were allowed to establish for one to two weeks before inoculation. Each plant, except for the water control, was inoculated with 20,000 *M. incognita* eggs/3.4 L soil. After 10 days chemical treatments were applied. The spirotetramat (Movento) study treatments included: i) Movento (0.42 and 0.63 kg ai/ha) plus MES-100 adjuvant (2.6ml/L) ii) water control, iii) nematode control, and iv) adjuvant control (Drexel, MES-100). Movento, mixed with an adjuvant at 2.6 ml/L, was foliar applied to each plant at both treatment rates as recommended from the Movento label. No treatment was applied to the water control pots. The water control was evaluated as a non-nematode treatment and used as a comparison for possible plant growth differences.

The MCW-2 study treatments included: i) MCW-2 (0.014 kg ai/ha) ii) water control, and iii) nematode control. MCW-2 was applied as a drench application to each pot by making 4 holes in the soil surface (10-cm-deep) and applying the solution. The MCW-2 rate (0.014 kg ai/ha) was converted from a volumetric rate of 4 mg ai/L of soil. This translated into 14 mg of ai applied per pot. Each treatment was replicated six to eight times in a randomized complete block design. Soil samples were collected ~ 40 and ~70 days after inoculation (DAI). At 40 DAI the soil was assayed using four soil cores (2.5-cm- diam × 15-cm-deep) and combined into one sample. Number of infective-stage juveniles (J2) were counted following extraction from a 100

cm^3 subsample of soil using soil sieves and the centrifugal-flotation technique (Jenkins, 1964). Roots were processed for *M. incognita* eggs using a 0.5% NaOCl solution (Hussey and Barker, 1973). The number of J2 in soil and eggs extracted from the respective root system were quantified and analyzed together in a total root-knot nematode value (RKN). At 70 DAI the remaining peach seedlings were destructively sampled. A 100 cm^3 soil sample was collected for determination of J2 population densities, and then an estimate of the total J2 count per pot was calculated. Foliage and shoots were removed and placed into paper bags and dry weights recorded. The roots were washed free of soil and saved for egg extraction and dry root weights. After processing the samples for J2's and eggs; the foliage, shoots, and roots were placed in an oven dryer set at 70 ºC and dry weights collected three to four days later. For the spirotetramat studies, samples were collected 49 and 85 DAI for the first study and 42 and 71 DAI for the replication study. The fluensulfone studies were sampled 43 and 70 DAI for the first study and 42 and 71 DAI for the replication study.

Ring nematode spirotetramat and fluensulfone studies: 'Nemaguard' peach seedlings were transplanted and established in the same manner as described above for the root-knot nematode studies. After establishment, each peach seedling, except for the water control, was inoculated with 1,000 *M. xenoplax* (all developmental stages)/3.4L soil equivalent to 30 nematodes/100cm^3 soil. After 10 days chemical treatments were applied. Treatments were the same as for the root-knot nematode study, except both chemicals were included in one study. The water control was evaluated as a non-nematode treatment and used as a comparison for possible plant growth differences. Each treatment was replicated six to seven times in a randomized complete block design. Soil samples were collected ~30, 60, and 90 DAI. At 30 and 60 DAI the soil was assayed using four soil cores (2.5-cm- diam × 15-cm-deep) from each pot

combined into one sample. From the sample 100 cm^3 of soil was collected and processed. Nematodes were extracted using the same techniques previously stated. Adult and juvenile *M. xenoplax* were counted under a stereomicroscope and population densities were then determined. At 90 DAI a 100 cm^3 sub-sample of soil was collected and assayed for *M. xenoplax* as previously described. Foliage and shoots then were removed and placed into paper bags and weights recorded. The roots were washed of soil and saved for root weights. After processing the samples for the ring nematode, the foliage, shoots, and roots were placed in a dryer at 70 ºC and dry weights collected three to four days later. Samples were collected 38, 60, 90 DAI for the first study and 30, 62, 90 DAI for the replication study.

Dual spirotetramat root-knot and ring nematode studies: Two separate studies using a dual application of Movento were also conducted with *M. incognita* and *M. xenoplax* . Due to a label application restriction limit of 1.05 kg ai/ha/season, only two applications were evaluated. Protocols were similar to the previous studies, with the first Movento and adjuvant application occurring 10 DAI followed by a second application at 40 DAI. The treatments for both root-knot and ring nematode studies included: i) Movento (0.42 and 0.63 kg ai/h) (two applications), ii) water control, iii) nematode control, and iv) (MES-100) adjuvant control (two applications). Soil samples were collected 40 and 70 days after inoculation for the root-knot nematode studies and 30, 60, and 90 DAI for the ring nematode studies. The root-knot nematode studies were terminated 70 DAI and the ring nematode studies at 90 DAI. Samples for the root-knot studies were gathered 42 and 70 DAI for the first study and 54 and 84 DAI for the replication study. The ring nematode studies were assayed 30, 63, 95 DAI for the first study and 30, 58, 90 DAI for the replication study.

Bioassay studies: The laboratory bioassay was conducted in 24-well plates to evaluate the efficacy of two chemical products on *M. incognita* J2 and *M. xenoplax* at room temperature (25 ± 2°C). The bioassay was comprised of four treatments; two foliar rates of Movento (0.42 and 0.63 kg ai/ha), one drench rate of MCW-2 (0.014 kg ai/ha), and a nematode control using sterile tap water. Rates used for these specific treatments were determined from the most efficacious rates developed from the greenhouse studies. A solution of 1 ml of each treatment was placed in each well and a 1 ml suspension of approximately 1,000 *M. xenoplax* (all developmental stages) were added to each well to attain the preferred concentration of active ingredient (a.i.) for all the chemical treatments. For the *M. incognita* studies, a 1 ml suspension of 500 J2 was made for the first bioassay and 1,000 J2 were used for repeat bioassay, since more J2 inoculum was available. The percentage of nematode mortality was determined 24, 48, and 72 hours after initial exposure to the treatments. To determine nematode mortality a 500 µl sub-sample from each treatment replication was pipetted from all 24 wells. The solutions were mixed thoroughly before extraction of each sub-sample. The sub-samples were then placed in 5-cm-diameter glass dishes containing 3 ml of autoclaved tap water, and were allowed to diffuse into the solution for one hour. The percentage of nematode mortality was determined by counting numbers of all intact, moving nematodes and non-motile nematodes under a stereomicroscope. The non-motile nematodes were considered alive if there was a response to prodding with a fine probe. Each bioassay was repeated once for each nematode species.

Statistical analysis: Nematode and egg counts for each treatment were transformed using $\log_{10}$, analyzed using one-way ANOVA, and means separated using Fisher's combined probability test. For all studies a two-way ANOVA analysis was conducted to evaluate

interactions between trials and if no significant interaction was detected data were combined for analysis.

RESULTS

Root-knot nematode spirotetramat and fluensulfone studies: The lower rate of spirotetramat at 0.42 kg ai/ha reduced ($P \leq 0.05$) the population of *M. incognita* in soil (J2) and roots (eggs) compared to the nematode control at 40 DAI. At 70 DAI, nematode population densities did not differ among treatments. No effects on nematode population densities were detected for spirotetramat at 0.63 kg ai/ha at 40 or 70 DAI (Table 1). Fluensulfone (0.014 kg ai/ha) was effective in lowering ($P \leq 0.05$) the *M. incognita* population compared to the nematode control at 40 DAI. However, like the lower rate 0.42 kg ai/ha of spirotetramat, the nematode suppressive effect of fluensulfone was lost at 70 DAI (Table 2). The adjuvant control was analogous to the nematode control with no distinction between *M. incognita* populations sampled (Table 1). No plant growth differences as measured by the dry weights were observed among treatments for both studies. No differences for root-knot nematode/gram of dry root were detected among the different treatments.

Ring nematode spirotetramat and fluensulfone studies: Both the low (0.42 kg ai/ha) and high (0.63 kg ai/ha) rates of spirotetramat were ineffective in suppressing population densities of the *M. xenoplax* population compared to the nematode control at 30, 60, and 90 DAI. However, fluensulfone was effective in suppressing ($P \leq 0.05$) the *M. xenoplax* population as compared to the nematode control at 30, 60, and 90 DAI (Table 3). No plant growth differences were observed among treatments for both studies.

Dual spirotetramat root-knot nematode studies: For the dual application of spirotetramat at the lower rate (0.42 kg ai/ ha), *M. incognita* population densities were significantly lower ($P \leq$

0.05) compared to the nematode control at 40 DAI (Table 4). However, like with the single application studies this suppression was not detected in the second sampling at 70 DAI, 30 days after the second application, as compared to the nematode control. Unlike the results observed in the single application studies, the application of spirotetramat at the higher rate of (0.63 kg ai/ ha) significantly ($P \leq 0.05$) reduced the *M. incognita* population densities compared to the nematode control at 40 DAI. This effect, like in the previous studies, did not appear in the final sampling at 70 DAI, for the higher rate of spirotetramat. The dual application of the adjuvant control showed no difference when evaluated against the nematode control. No plant growth differences were observed among treatments for both studies. No differences for root-knot nematode/g dry root weight were observed among treatments.

Dual spirotetramat ring nematode studies: As was observed in the single application studies the lower rate of spirotetramat at 0.42 kg ai/ha applied twice was ineffective in suppressing *M. xenoplax* reproduction compared to the nematode control at 30, 60, and 90 DAI. The second application at the higher rate of spirotetramat at 0.63 kg ai/ha was also similar with no significant decrease in *M. xenoplax* population at 30, 60, or 90 DAI, compared to the nematode control. The dual application of the adjuvant control showed no difference when evaluated against the nematode control (Table 5). No plant growth differences were observed among treatments for both studies.

M. xenoplax bioassay: In the first *M. xenoplax* bioassay at the 24 hour observations both rates of spirotetramat (0.42 and 0.63 kg ai/ha) suppressed mobility compared to the untreated control. Similar treatment effects were observed at the 48 and 72 hour samplings. Fluensulfone 0.014 kg ai/ha significantly decreased *M. xenoplax* mobility to a lower level than both rates of spirotetramat and the untreated control at all three sampling times; 24, 48, 72 hours. In the

second *M. xenoplax* bioassay, at 24 hours both rates of spirotetramat significantly suppressed nematode mobility compared to the control; with greater suppression occurring at the 0.63 kg ai/h rate than at the 0.42 kg ai/h rate. Fluensulfone significantly decreased ring nematode mobility and was similar to the higher rate of spirotetramat when compared to the control treatment. At the 48 hour sampling, both rates of spirotetramat and fluensulfone suppressed ring nematode mobility compared to the untreated control. At the 72 hour sampling both rates of spirotetramat suppressed ring nematode mobility compared to the control, whereas fluensulfone significantly decreased ring nematode mobility at a lower level than both rates of spirotetramat and the untreated control (Table 6).

Root-knot nematode bioassay: In the first and second bioassay studies neither rate of spirotetramat (0.42 and 0.63 kg ai/h) was found to be efficacious in significantly reducing J2 mobility at the 24, 48, 72 hour sampling times compared to the control. In contrast, fluensulfone (0.014 kg ai/h) significantly reduced J2 mobility on all three sampling times compared to both spirotetramat treatments and the untreated control (Table 7).

DISCUSSION

Both spirotetramat and fluensulfone were evaluated for the control of ring and root-knot nematode on peach in the greenhouse. The goal of this research is to provide the peach industry with new post-plant nematicides for control of ring and root-knot nematodes in peach. Post-plant control is absolutely essential to extend the life of peach trees on PTSL sites, since the nematode populations increase and re-establish in the years subsequent to pre-plant fumigation.

Root-knot Nematode Spirotetramat and Fluensulfone Studies: For both chemical studies similar results became apparent. With the spirotetramat single application studies only the lower rate of spirotetramat 0.42 kg ai/ha significantly suppressed the *M. incognita* population when

compared to the nematode control after the first generation and sampling. After the second generation and sampling at 70 DAI this difference was lost for both trials. For these trials, chemicals were applied to the plants 10 days after inoculation. At this point in time most of the viable J2's should have entered the roots and been in contact with the chemical. Some of the root-knot egg inoculum would not have hatched immediately and therefore could have entered the roots after the effectiveness of the product had dissipated. Movento has been shown to be active within the roots for two or more weeks (Bruck et al. 2009; Smiley et al. 2011, 2012). At the same time spirotetramat is known to reduce fecundity and fertility of the organism, but has not shown nematicidal activity (Bruck et al. 2009). Given this level of activity, some of the nematodes were still reproducing and re-infecting the host. This response along with non-synchronous hatching may explain the loss of treatment effects at the second generation sampling. Also, due to the pot size and growth of the plants it became apparent two generations would be the limit for our greenhouse studies. Field studies are needed to determine long-term effects of these products.

The fluensulfone studies had similar results, with a drench application of MCW-2 effectively reducing the *M. incognita* population densities compared to the nematode control. Like in the spirotetramat studies, the efficacy did not carry over into the second generation. MCW-2 was drench applied and has been shown in previous studies to have a nematicidal effect and is partially root systemic (Everich and Schiller, 2009), which may help explain the results obtained in the root-knot nematode studies. Like with the spirotetramat studies MCW-2 was applied 10 DAI. Any J2's within the roots would not have come in contact with this chemical, unless via the vascular system, and would have begun development and reproduction. Those J2's that were delayed in hatching would be in direct contact with the chemical, having an opposite

effect on *M. incognita* inoculum as the spirotetramat studies, but have similar population results at 40 DAI. The exact reason the effect diminished at 70 DAI is not known, but one explanation may be due to a delay in root-knot development, meaning that egg-laying and resulting J2 hatch were delayed beyond the 40 DAI assays.

Ring nematode spirotetramat and fluensulfone studies: For the ring nematode studies we had different results than with *M. incognita* for each chemical. Given that spirotetramat is foliar applied and transported to the apical portions of the plant, it is possible that this chemical was limited to the vascular column of the roots (Bruck et al. 2009). This limitation would have an obvious effect on the sedentary endoparasitic root-knot nematode, which feeds on vascular tissue. On the other hand the ectoparasitic ring nematode feeds on cortex root tissue and not the vascular column (Hussey et al. 1992). Therefore, if spirotetramat is limited to the vascular column of the root and does not readily pass through the pericycle into the root cortex where the ring nematode feeds, resulting suppression would be minimal.

For the same reason spirotetramat was ineffective, MCW-2 worked quite well and may help to explain the drastic drop in *M. xenoplax* population by the drench application. The ring nematode is an ectoparatic nematode and is always in contact with the soil rhizosphere. In this zone, water, nutrients, metabolites, and also chemicals like MCW-2 accumulate (McNear, 2013). Thus, the ring nematode would be in constant contact with MCW-2 until the chemical starts to degrade. MCW-2 is believed to be partially root systemic meaning it would move into the cortex, but would likely not pass the pericycle into the vascular system (Everich and Schiller, 2009). This should help to explain why fluensulfone was able to significantly reduce *M. xenoplax* population densities at all sampling dates.

Dual Movento Root-knot Nematode Studies: The reason both rates of spirotetramat were effective at 40 DAI for this study and not the single application study is unknown. A possible explanation could be the peach seedlings in these studies were better able to transport the chemical to the root. It is also likely the root-knot nematode inoculum was affected by the time of year and resulting effects on greenhouse conditions. Each sampling date was pushed forward around two weeks, due to delay in the normal 30-day life cycle, for the second trial. This delay could explain the clearer results for the 40 DAI sampling. What is still unclear is why the second application at 40 DAI had no effect on *M. incognita* population densities at 70 DAI. Possibly the chemical has more of an effect on the juveniles and less so with the adults (Smiley et al. 2011, 2012).

Dual Movento Ring Nematode Studies: In both trials, we were unable to detect any differences in ring nematode populations among treatments. This lack of nematode suppression may be due to *M. xenoplax* feeding habit. As previously mentioned, spirotetramat is transported to the apical portions of the plant roots via the foliage. The ring nematode, which feeds on the root cortex cells, may not come in contact with spirotetramat, which would accumulate in the vascular root tissue. Given that spirotetramat works well against the root-knot nematode, this chemical may therefore be more efficacious against nematodes that feed and come in contact with the root's vascular system.

Root-knot nematode bioassay: Spirotetramat had no effect in terms of J2 mobility. This lack of response may be because the chemical needs to be ingested by the nematode and cannot easily move through the cuticle of the root-knot nematode. Fluensulfone, on the other hand, worked quite well in suppressing J2 mobility at all sampling times. Though the mode of action is not currently known, more than likely this chemical has the ability to penetrate the cuticle of the

root-knot nematode, based on laboratory observation, and reduce J2 mobility, with greater than 90% suppression for each test.

Ring nematode bioassay: The *M. xenoplax* bioassays showed differing results than the *M. incognita* bioassay. For these tests both chemicals worked well in suppressing the nematode mobility. The reason spirotetramat at both rates was able to penetrate the nematode cuticle to reduce mobility at all sampling times is unknown. Possibly the ring nematode was more active in diffusing the contents of the micro-wells than the root-knot nematode, thus more exposure to the chemicals. Since the mode of action for fluensulfone is unknown but it worked well in reducing ring nematode mobility at all sampling times.

In summation, we showed the potential of the post-plant nematicides, spirotetramat and fluensulfone, for use in the control of *M. incognita* and *M. xenoplax* in peach. The peach industry in the southeastern US, including other major commodities, has long been in need of a viable replacement for soil fumigants and/or additional option for control of plant-parasitic nematodes. This research is a promising step in the right direction in terms of providing producers with another practical management strategy. More work will need to be conducted to better understand spirotetramat and fluensulfone's effect in the orchard and over a longer period of time.

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TABLES

Table 1. Effect of spirotetramat on *Meloidogyne incognita* reproduction on 'Lovell' peach in the greenhouse from two studies data combined.

	RKN/ 100cm^3 soil[a]		RKN/pot[b]		RKN/g dry root system[c]	
Treatment	40 DAI		70 DAI		70 DAI	
NC	1162	a[d]	70250	a	13147	a
ADJ	1004	a	50966	a	11253	a
SPT(0.63kg)	665	ab	51101	a	7173	a
SPT(0.42kg)	443	b	32729	a	5862	a

Data are means of 14 replications [Study 1 (8 replication) and Study 2 (6 replications)].
[a] RKN/100 cm^3 soil = number of *M. incognita* J2 per 100 cm^3 soil combined with number of eggs extracted from root segments obtained from 100 cm^3 soil subsample.
[b] Total number of J2 and number of eggs per root system.
[c] Total RKN per plant divided by total dry root weight.
[d] Treatments include; nematode control (NC), adjuvant [ADJ (= MES-100)], and spirotetramat (SPT 0.63 kg and 0.42 kg respectively). Adjuvant and spirotetramat were applied 10 days after inoculation (DAI).
[e] Initial population density of *M. incognita* = 667 eggs/100 cm^3 soil.
[f] Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 2. Effect of fluensulfone on *Meloidogyne incognita* reproduction on 'Lovell' peach in the greenhouse from two studies data combined.

	RKN/ 100cm^3 soil[a]		RKN/pot[b]		RKN/g dry root system[c]	
Treatment	40 DAI		70 DAI		70 DAI	
NC	1162	a[d]	139697	a	20253	a
FLU(0.014kg)	268	b	102292	a	12221	a

Data are means of 12 replications [Study 1 (6 replication) and Study 2 (6 replications)].
[a] RKN/100 cm^3 soil = number of *M. incognita* J2 per 100 cm^3 soil combined with number of eggs extracted from root segments obtained from 100 cm^3 soil subsample.
[b] Total number of J2 and number of eggs per root system.
[c] Total RKN per plant divided by total dry root weight.
[d] Treatments include; nematode control (NC), fluensulfone (FLU 0.014 kg). Fluensulfone applied 10 DAI
[e] Initial population density of *M. incognita* = 667 eggs/100 cm^3 soil.
[f] Means within the columns followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 3. Effect of fluensulfone and spirotetramat on *Mesocriconema xenoplax* reproduction on 'Nemaguard' peach in the greenhouse from two studies data combined.

	Nematodes/ 100cm3 of soil[a]					
Treatment	30 DAI		60 DAI		90 DAI	
NC	75	a[b]	194	a	1557	a
ADJ	137	a	138	a	1127	a
SPT(0.63kg)	85	a	198	a	1071	a
SPT(0.42kg)	84	a	99	a	1441	a
FLU(0.014kg)	12	b	11	b	65	b

Data are means of 12 replications [Study 1 (6 replication) and Study 2 (6 replications)].
[a] Total ring nematode count, all life stages, per 100 cm^3 soil.
[b] Treatments include; nematode control (NC), adjuvant [ADJ (= MES-100)], spirotetramat (SPT 0.63 kg and 0.42 kg respectively), and fluensulfone (FLU 0.014 kg). Adjuvant, fluensulfone, and spirotetramat were applied 10 DAI.
[c] Initial population density of *M. xenoplax* 33 nematodes/100 cm^3 soil.
[d] Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 4. Effect of dual applications of spirotetramat, 10 & 40 DAI, on *Meloidogyne incognita* reproduction on 'Lovell' peach in the greenhouse from two studies data combined.

	RKN/ 100cm^3 soil[a]		RKN/pot[b]		RKN/g root system[c]	
Treatment	40 DAI		70 DAI		70 DAI	
NC	485	a[d]	12869	a	1672	a
ADJ	503	a	7440	a	1596	a
SPT(0.63kg)	221	b	3427	a	328	a
SPT(0.42kg)	203	b	6788	a	1301	a

Data are means of 14 replications [Study 1 (6 replication) and Study 2 (8 replications)].
[a] RKN/100 cm^3 soil = number of *M. incognita* J2 per 100 cm^3 soil combined with number of eggs extracted from root segments obtained from 100 cm^3 soil subsample.
[b] Total number of J2 and number of eggs per root system.
[c] Total RKN per plant divided by total dry root weight.
[d] Treatments include; nematode control (NC), adjuvant [ADJ (= MES-100)], and spirotetramat (SPT 0.63 kg and 0.42 kg, respectively). Adjuvant and spirotetramat were applied 10 & 40 DAI.
[e] Initial population density of *M. incognita* = 667 eggs/100 cm^3 soil.
[f] Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 5. Effect of dual applications of spirotetramat, 10 & 40 DAI, on *Mesocriconema xenoplax* reproduction on 'Nemaguard' peach in the greenhouse from two studies data combined.

	Nematodes/ 100cm3 of soil[a]		
Treatment	30 DAI	60 DAI	90 DAI
NC	170 a[b]	52 a	295 a
ADJ	216 a	56 a	385 a
SPT(0.63kg)	163 a	54 a	346 a
SPT(0.42kg)	189 a	73 a	391 a

Data are means of 12 replications [Study 1 (6 replication) and Study 2 (6 replications)].
[a] Total ring nematode count, all life stages, per 100 cm^3 soil.
[b] Treatments include; nematode control (NC), adjuvant [ADJ (= MES-100)], and spirotetramat (SPT 0.63 kg and 0.42 kg respectively). Adjuvant and spirotetramat were applied 10 & 40 DAI.
[c] Initial population density of *M. xenoplax* 33 nematodes/100 cm^3 soil.
[d] Means within the columns with the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 6. Laboratory bioassay for the comparison of spirotetramat and fluensulfone on *Mesocriconema xenoplax* mobility, two studies data combined.

	% Motile Nematodes/ 200µL of solution[a]					
	Test 1			Test 2		
Treatment[b]	24 Hrs	48 hrs	72 hrs	24 Hrs	48 hrs	72 hrs
NC	52.9 a[c]	48.9 a	60.1 a	56.9 a	44.8 a	56.2 a
SPT(0.42kg)	36.3 b	32.1 b	43.3 b	47.8 b	30.5 b	35.3 b
SPT(0.63kg)	33.2 b	24.7 b	34.0 b	32.4 c	32.1 b	32.4 b
FLU(0.014kg)	17.1 c	8.05 c	4.79 c	28.9 c	25.5 b	19.2 c

Data are means of 12 replications [Study 1 (6 replication) and Study 2 (6 replications)].
[a] % motile J2 nematodes per 200µL of solution.
[b] Treatments include; nematode control (NC), adjuvant (ADJ), spirotetramat (SPT 0.63 kg and 0.42 kg respectively), and fluensulfone (FLU(0.014 kg)).
[c] Means within the columns with the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 7. Laboratory bioassay for the comparison of spirotetramat and fluensulfone on *Meloidogyne incognita* J2 mobility, two studies data combined.

	% Motile J2/ 200µL of solution[a]					
	Test 1			Test 2		
Treatment[b]	24 Hrs	48 hrs	72 hrs	24 Hrs	48 hrs	72 hrs
NC	94.0 a[c]	90.9 a	94.7 a	98.8 a	99.3 a	99.5 a
SPT(0.42kg)	94.8 a	94.0 a	96.5 a	98.1 a	98.5 a	99.7 a
SPT(0.63kg)	93.0 a	93.9 a	91.6 a	98.7 a	98.4 a	99.7 a
FLU(0.014kg)	48.2 b	46.2 b	11.0 b	65.6 b	8.56 b	2.23 b

Data are means of 12 replications [Study 1 (6 replication) and Study 2 (6 replications)].
[a] % motile J2 per 200µL of solution.
[b] Treatments include; nematode control (NC), adjuvant (ADJ), spirotetramat (SPT 0.63 kg and 0.42 kg respectively), and fluensulfone (FLU(0.014 kg)).
[c] Means within the columns with the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

CHAPTER 4

UTILIZATION OF GA534 FOR THE CONTROL OF *MELOIDOGYNE INCOGNITA* ON PEACH

Shirley, A.M., Nyczepir, A.P., Noe, J.P., and P.M. Brannen. To be submitted to *Journal of Nematology*.

Abstract: A series of greenhouse trials were used to determine the efficacy of a biologically derived nematicide, GA534, against the root-knot, *Meloidogyne incognita*, nematode on Peach (*Prunus persica* (L.) Batsch). GA534 was evaluated for its effect on *M. incognita* reproduction and plant weights were taken to determine any growth differences among treatments. The treatments included; GA534 at 300 and 500 ml/20-cm-diameter pot, and a nematode control with no treatment applied. At 40 days after inoculation (DAI) in the first trial both rates of GA534 significantly reduced *M. incognita* population densities compared to the nematode control. At 70 DAI no effect was observed for GA534 on *M. incognita* population densities at 500 ml or 300 ml, compared to the nematode control. For the second trial, only GA534 at (300 ml/pot) was used. At 40 DAI, GA534 was effective in suppressing *M. incognita* populations compared to the nematode control. At 70 DAI no effect was observed for GA534 on *M. incognita* populations compared to the nematode control. No plant growth differences were observed among treatments.

Key words: Biological control, GA534, management, *Meloidogyne incognita*, peach, *Prunus persica*, rootknot nematode.

Peach production in the southeastern United States dates back to the late 1600s, and reports of peach replant issues and disease in orchards are just as old (Brittain and Miller, 1978). The Southeast, particularly Georgia, has long been known for its peach production. This is partly due to the regions favorable climate, soil types, and market availability. Despite the success of peach production in the Southeast, the number of planted trees has decreased drastically over the last 10 years (2002 to 2012) from 150,000 acres to 120,000 acres. Much of this is attributed to the impact of nematodes associated with Peach Tree Short Life (PTSL) or peach tree decline diseases (e.g., Armillaria root rot), and environmental factors leading to a reduction in peach tree survival and productivity. In recent years, disease management and nematode control options for producers have become much more difficult. Producers are dealing with the loss of methyl bromide, the increased cost of fumigants [e.g., Telone II (1, 3-D)], and possibly the eventual loss of tolerance in current rootstocks, due to nematode diversity. There is an apparent need for new management/cultural practices and alternative chemicals for controlling peach nematode pathogens which will provide the producer with optimum productivity.

Peach tree decline, unlike PTSL, is associated with the root-knot nematode and the root-lesion nematode (*P. vulnus*) (Nyczepir, 2011; Ritchie and Clayton, 1981). The root-knot nematodes are by far the most damaging and prevalent plant-parasitic nematodes in the world and are found in all agricultural production areas ranging from temperate to tropical climates (Lamberti, 1979; Sasser, 1979; Sasser and Freckman, 1987). The root-knot nematodes, *M. incognita* and *M. javanica* were found in 95% and 5% of peach orchards surveyed in South Carolina, respectively (Nyczepir et al. 1997).

As a plant-parasitic nematode on peach, the root-knot nematode's aboveground symptoms include a reduction in fruit yield and plant growth, and promotion of early defoliation

in severely stunted plants. Belowground symptoms include; a reduced root system with malformation and galling present. Under severe conditions these symptoms can even lead to tree death (Nyczepir et al. 1993). Since the root-knot nematode is an obligate sedentary endoparasitic the use of post-plant nematicides applied to the tree or to the root zone should offer a useful control option.

GA534 is a biologically derived nematicide developed and evaluated for the control of root-knot nematode, *M. incognita,* in cotton. The product is a fungal culture filtrate obtained from the GA534 isolate (species is confidential), and has been shown to significantly suppress root-knot nematode reproduction 120 days after planting when applied as a soil drench at the base of growing cotton plants. Evaluation of this product was conducted at four different cotton field sites in Georgia in 2009. Results indicate that there was approximately a 55% reduction in *M. incognita* J2 and egg population in plots treated with GA534 (Noe, 2009). If GA534 is successful it will provide an improved post-plant control strategy of peach nematode pathogens for peach growers to utilize in the Southeast. This product is naturally derived and environmentally-friendly and if given proper certification could provide organic producers with a useful nematicide.

MATERIALS AND METHODS

Nematode source and inoculum: A population of *M. incognita* was isolated from peach in Georgia, and maintained on eggplant (*Solanum melongena* cv. 'Black Beauty') in a greenhouse culture at the University of Georgia, Athens. Eggs of *M. incognita* were collected from eggplant roots using a 0.5% NaOCl solution (Hussey and Barker, 1973).

'Lovell' peach seedlings were transplanted into 20-cm-diameter standard clay pots containing 3.4 L sterilized loamy sand soil mixture of 25% field soil, 50% sand, and 25%

Fafard® germinating mix and placed on benches in a greenhouse (~ 27 ± 5° C). After transplanting, ~1 ml (1/4 teaspoon) of 13-13-13 was applied to each pot and water was applied as needed. Each plant was allowed to establish for one to two weeks before inoculation. Each plant was inoculated with 20,000 *M. incognita* eggs/3.4 L soil. After 10 days chemical treatments were applied. The GA534 study treatments included: i) GA534 at 300 ml/pot, ii) GA543 at 500 ml/pot, and iii) a nematode control with no treatment applied. GA534 was started in the lab using a pure culture of the fungus. To extract GA534, potato dextrose broth cultures were established using the fungal culture. Four pugs were taken from the culture and placed into sterile autoclaved broth media and allowed to grow for approximately one month in an orbital platform shaker. After which time the broth media was strained through cheese cloth to remove large solids. The resulting broth/fungal extract mixture was used for each trial. GA534 was applied in a 50% dilution with H_2O to each pot at both rates. At 40 DAI the soil was assayed using four soil cores (2.5-cm- diam × 15-cm-deep) and combined into one sample. Number of infective-stage juveniles (J2) were counted following extraction from a 100 cm^3 subsample of soil using soil sieves and the centrifugal-flotation technique (Jenkins, 1964). Root fragments collected on the sieve were processed for *M. incognita* eggs using a 0.5% NaOCl solution (Hussey and Barker, 1973). The number of J2 and eggs were counted under a stereomicroscope. At 80 DAI in the first trial and 68 DAI in the second trial, a 100 cm^3 soil sample was collected from each pot to determine the population density of J2 nematodes, and then the total population density of J2 nematodes per pot was calculated. Foliage and shoots were removed and placed into paper bags and dry weights recorded. The roots were washed free of soil and saved for egg extraction and dry root weights. After processing the samples for J2's and eggs; the foliage, shoots, and roots were placed in an oven dryer at 70 ºC and dry weights collected three to four days later*Statistical*

analysis: Nematode and egg counts for each treatment were transformed using $\log_{10}(x+1)$, analyzed using one-way ANOVA, and means separated using Fisher's combined probability test.

RESULTS

For the first trial both rates of GA 534, 300 and 500 ml, were effective in suppressing *M. incognita* population densities at 40 DAI compared to the nematode control. At 70 DAI neither rate significantly suppressed the *M. incognita* population densities compared to the nematode control (Table 1). No plant growth differences as measured by the dry weights were observed among treatments. No differences were observed for root-knot nematode/ gram of dry root weight among each treatment.

For the second trial GA534 at 300 ml significantly reduced *M. incognita* population densities at 40 DAI compared to the nematode control. At 70 DAI GA534 no differences in *M. incognita* population densities were observed for treatment with GA534 compared to the nematode control (Table 2). No plant growth differences as measured by the dry weights were observed among treatments. No differences for root-knot nematode/ gram of dry root weight among each treatment.

DISCUSSION

GA534 is a promising alternative for control of *M. incognita* on peach. Both the 300 and 500 ml rates were effective at 40 DAI in suppressing *M. incognita* population densities in the first trial, but not at 70 DAI. The 300 ml rate only was repeated in a second trial due to the higher rate, 500 ml, possibly having a phytotoxic effect on peach. This phytotoxicity was not proven by plant weights, only on greenhouse observation of the 500 ml treated plants having less foliage with visually less root growth. The mode of action of GA534 in control of *M. incognita* in the soil is currently unknown. It’s speculated to be active when it’s taken up by the plant and

ingested by the J2 nematodes, which have newly penetrated the root, with subsequent effects on development and fecundity of adult females. Bioassay work in the nematology lab, in Athens, has shown that GA534 does not negatively impact J2 viability in solution (Noe, verbal communication). Future field work with GA534 on peach should be done to further supplement these results.

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TABLES

Table 1. Effect of GA534 on *Meloidogyne incognita* (RKN) reproduction on 'Lovell' peach in the greenhouse, first trial.

Treatment	RKN/ 100cm^3 soil[a] 40 DAI		RKN/pot[b] 70 DAI		RKN/g dry root system[c] 70 DAI	
NC	427	a[e]	800	a	13	a
300 ml	55	b	507	a	8	a
500 ml	0	b	396	a	7	a

Data are means of 6 replications

[a] RKN/100 cm^3 soil = number of *M. incognita* J2 per 100 cm^3 soil combined with number of eggs extracted from root segments obtained from 100 cm^3 soil subsample.

[b] Total number of J2 and number of eggs per root system.

[c] Total RKN per plant divided by total dry root weight.

[d] Treatments include; nematode control (NC), 300 ml (GA534), and 500 ml (GA534).

[e] Means within the columns followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

Table 2. Effect of GA534 on *M. incognita* (RKN) reproduction on 'Lovell' peach in the greenhouse, second trial.

	RKN/ 100cm^3 soil[a]		RKN/pot[b]		RKN/g dry root system[c]	
Treatment	40 DAI		70 DAI		70 DAI	
NC	3253	a[e]	122303	a	9921	a
300 ml	616	b	64510	a	3414	a

Data are means of 6 replications

[a] RKN/100 cm^3 soil = number of *M. incognita* J2 per 100 cm^3 soil combined with number of eggs extracted from root segments obtained from 100 cm^3 soil subsample.

[b] Total number of J2 and number of eggs per root system.

[c] Total RKN per plant divided by total dry root weight.

[d] Treatments include; nematode control (NC), 300 ml (GA534), and 500 ml (GA534).

[e] Means within the columns followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.

CHAPTER 5

HOST STATUS OF TALL FESCUE ‘JESUP (MAX-Q)’ TO THE PEACH ROOT-KNOT NEMATODE, *MELOIDOGYNE FLORIDENSIS*

Shirley, A.M., Nyczepir, A.P., Noe, J.P., and P.M. Brannen. To be submitted to *Journal of Nematology*.

Abstract: Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumont. cv. Jesup (Max-Q)] was recently assessed for its susceptibility to *Meloidogyne floridensis* in a series of greenhouse trials. For these trials the host status of Jesup (Max-Q), hereafter referred to as Max-Q, was evaluated against *M. floridensis*, *M. incognita* (known nonhost), and *M. arenaria* (known host). For comparison, tomato 'Rutgers' a good host to all *Meloidogyne* spp. served as a control treatment. The study was conducted three times with differing results. In trial 1, Max-Q was effective in suppressing reproduction of all *Meloidogyne* species. For trials 2 & 3, Max-Q served as a poor host to both *M. floridensis* and *M. incognita*, but was effective in only suppressing reproduction of *M. arenaria*. Due to contradicting results based on previous studies with Max-Q, more work will need to be completed to better understand the 'true' susceptibility of Max-Q to the peach root-knot nematode, *M. floridensis*.

Key words: Endophyte, host-parasite relationship, management, *Meloidogyne arenaria*, *Meloidogyne floridensis*, *Meloidogyne incognita*, resistance, root-knot nematode, *Schedonorus arundinaceus*, tall fescue grass.

Management of the root-knot nematode is an ongoing battle with resistance, multiple plant hosts, *Meloidogyne* spp. races, and proper chemical controls. The root-knot nematodes are by far the most damaging and prevalent plant-parasitic nematodes in the world and are found in all agricultural production areas ranging from temperate to tropical climates (Lamberti, 1979; Sasser, 1979; Sasser and Freckman, 1987). The root-knot nematodes, *M. incognita* and *M. javanica* were found in 95% and 5% of peach orchards surveyed in South Carolina, respectively (Nyczepir et al. 1997). A newly identified nematode *M. floridensis*, the peach root-knot nematode, previously described as *M. incognita* (Handoo et al. 2004), has been shown to parasitize the resistant peach rootstocks Nemaguard and Guardian®, which are both known to be resistant to *M. incognita* and *M. javanica*. Currently the only peach rootstocks with known resistance to *M. floridensis* are Flordaguard, MP29, and Sharpe (Beckman et al. 2012; Beckman et al. 2008; Nyczepir et al. 2006). This nematode is only found in seven contiguous counties in Florida, though *M. floridensis* could potentially pose a major economic issue to growers throughout the Southeast. *Meloidogyne floridensis* could easily be disseminated to other peach growing regions throughout the Southeast (Brito et al 2008; Brito et al. 2010).

As a plant-parasitic nematode on peach the root-knot nematode's aboveground symptoms include: a reduction in fruit yield, plant growth, and promotion of early defoliation in severely stunted plants. Belowground symptoms include; a reduced root systems with malformation and galling present. Under severe conditions these symptoms can even lead to tree death (Nyczepir et al. 1993).

The use of pre-plant rotation with groundcovers down peach tree rows could serve as a management practice to reduce plant-parasitic nematode populations and any associated disease. Currently in the Southeast when a peach orchard is removed due to severe stunting from root-

knot nematode damage or PTSL from ring nematode (*Mesocriconema xenoplax*), the practice is to apply pre-plant fumigation using Telone II along with a resistant rootstock to insure increased tree longevity and maximum nematode protection (Horton et al. 2013). In recent years peach growers affected by economic recession have found it difficult to afford the costs associated with pre-plant fumigation and/or are unable to apply the fumigants at the proper time of the year due to management conflicts with other crops. More commonly in the Southeast, when a peach orchard is removed due to damage from PTSL or root-knot nematode damage, the grower decides not to replant the old site back to peaches. Often these sites are planted in small grains or some other cash crop, due to the trouble of regaining the field from ring or root-knot nematodes. These issues have led to a growing interest in the use of groundcovers as a nematode management strategy in peach producing areas. One crop which has shown to be a promising crop rotation option is the tall fescue grass cultivar Jesup (Max-Q). Max-Q is a non-toxic endophyte (*Neotyphodium coenophialum*) infested tall fescue developed as a viable forage crop for cattle production in eastern USA and some areas in the West. Its growing popularity among producers is due to the presence of this novel fungal endophyte that does not produce ergot alkaloids that cause fescue toxicosis, but does impart drought tolerance that is greatly needed (Phillips et al., 2009). Max-Q has been shown as a non-host/poor host to a number of nematodes. In a recent study the host status of Max-Q was tested against four *Meloidogyne* spp. It was determined Max-Q is a non-host to *M. incognita* and *M. hapla*, a poor host for *M. javanica* and a good host for *M. arenaria* (Nyczepir and Meyer, 2010). Also the host status of Max-Q was determined for *M. xenoplax* and *P. vulnus*. It was shown Max-Q is a poor host to *P. vulnus*, but a good host to *M. xenoplax* (Nyzcepir, 2011). The host status of Max-Q to the newly described root-knot species *M. floridensis* and the length of time needed for rotation of this crop prior to

planting the orchard site back to peach have not yet been determined. Given the nonhost or poor host status of Max-Q to the nematodes mentioned above (i.e., *M. incognita*, *M. javanica*, and *P. vulnus*), this plant can potentially function as a good candidate for a pre-plant groundcover rotation strategy in suppressing these peach nematode pathogens in the southeastern USA.

MATERIALS AND METHODS

Nematode source and inoculum: The populations of *Meloidogyne floridensis*, *M. incognita*, and *M. arenaria* were all maintained on tomato (*Solanum esculentum* Mill. cv. 'Rutgers') in the greenhouse at the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory, Byron, Georgia. Tomato roots were processed for root-knot nematode egg inoculum using a 0.5% NaOCl solution (Hussey and Barker, 1973).

Host status of Max-Q to Meloidogyne spp.: Max-Q was evaluated for its host susceptibility to *M. floridensis* in the greenhouse. All trials were completed at the USDA-ARS SE Fruit and Tree Nut Research Laboratory in Byron, Georgia Rutgers tomato was included as a susceptible control for all three species in all trials. Five Max-Q seed or individual tomato seedlings were planted in 15-cm-diameter plastic pots filled with 1,500 cm^3 steam pasteurized loamy sand (86% sand, 10% silt, 4% clay, 0.54% organic matter). Approximately 30 days after planting, Max-Q seedlings were thinned to one plant per pot. Ten days after thinning the soil in each pot was infested with 3,000 *M. arenaria*, *M. floridensis*, or *M. incognita* eggs (Nyczepir et al., 1999; Nyczepir and Meyer, 2010). Approximately 1,500 eggs were pipetted directly into each of two holes (2.5 cm-deep), one on either side of the plant stem. The holes were covered and additional water applied to settle the potting medium around the eggs. Eight replications of each plant and nematode species were arranged in a randomized complete block with a split-plot design on benches in the greenhouse (24 ± 14ºC). All plants were fertilized with Osmocote (13-

13-13) and watered as needed. The experiment was terminated 75 days after inoculation and the following data were collected: total egg masses per root system (up to 101), the highest rating according to Taylor and Sasser (1978) egg mass index, number of eggs per root system, number of galls per root system (up to 101), and root dry weight (all root systems were dried at 70ºC in aluminum foil until no further weight loss occurred) (Nyczepir and Meyer, 2010). Nematode eggs were extracted from the root systems using a NaOCl solution as described above. The egg index system is based on a rating scale from 0 to 5, with 0 = no egg masses, 1 = 1 to 2 egg masses, 2 = 3 to 10 egg masses, 3 = 11 to 30 egg masses, 4 = 31 to 100 egg masses, 5 = >100 egg masses (Taylor and Sasser, 1978). Host susceptibility was determined using the egg mass index rating scale by Taylor and Sasser determined as: 0 = nonhost (highly resistant), 1-2 = poor host (resistant), and ≥ 3 = a good host (susceptible) (Nyczepir and Meyer, 2010).

The experiment was repeated two times with minor modifications, which included terminating the second trial and the third trial 76 and 74 days after inoculation, respectively.

Meloidogyne spp. tomato bioassay: After the first trial, soil from the Max-Q and tomato treatments, infested with *M. floridensis*, was kept separate and saved for use in a tomato bioassay. Soil by treatment was placed back into the 15-cm diameter plastic pots and a single Rutgers tomato was planted in each pot to assay the soil for viable *M. floridensis*. Seventy five days after planting the bioassay was taken down and the same data was recorded as stated above for the evaluation study. For the second trial a tomato bioassay was not conducted, but a soil bioassay was conducted for the third trial with all three of the nematode species included. The final bioassay was terminated 75 days after planting and data was collected in the same manner as the previous bioassay study.

Statistical analysis: All data collected were subjected to analysis of variance (ANOVA) and means separated using Fisher's combined probability test.

RESULTS

Meloidogyne spp. evaluation in Max-Q: In all three trials, tomato (known susceptible) sustained higher population densities ($P \leq 0.05$) of all *Meloidogyne* spp. than on Max-Q based on number of egg masses per plant and number of number of eggs/gram of dry root (data not shown). Comparable results were observed for number of root galls per plant. Reproduction among the three *Meloidogyne* spp. on Max-Q was similar in all trials (Table 1, 2, and 3). Host susceptibility of Max-Q, based on egg mass index, differed among the *Meloidogyne* spp. and the three experiments. Max-Q did not support *M. arenaria* reproduction and would be classified highly resistant (nonhost; 0 egg masses) in all three trials. For *M. incognita*, Max-Q was classified a nonhost (0 egg masses) in trials 1 and 2, but a poor host (4 egg masses) for trial 3. For *M. floridensis*, Max-Q was also classified a nonhost (0 egg masses) for trial 1, but a poor host (2 egg masses) in trials 2 & 3 ($P \leq 0.05$) (Table 1, 2, and 3).

Meloidogyne spp. tomato bioassay: In bioassay 1, tomato (known susceptible) supported greater ($P \leq 0.05$) reproduction of *M. floridensis* in soil previously planted to tomato than in soil previously planted to Max-Q based on number of egg masses per plant (90 vs. 0, respectively) and number of eggs/gram of dry root (264,477 vs. 20, respectively). Similar results were observed for number of root galls per plant (101 vs. 0, respectively). However, in bioassay 3, where all three *Meloidogyne* spp. were evaluated, only with *M. arenaria* were nematode population densities less ($P \leq 0.05$) on tomato in soil previously planted to Max-Q (Table 4). Population densities of *M. floridensis* and *M. incognita* on Max-Q bioassays indicated fairly high residual nematode populations on tomato in soil previously planted to Max-Q.

DISCUSSION

Meloidogyne spp. evaluation in Max-Q: For this study results indicate that Max-Q was classified as a nonhost for *M. arenaria* in all three trials and a poor host for *M. incognita* and *M. floridensis* in two out of three trials. The results for *M. incognita* and *M. arenaria* contradict previous findings reported by Nyczepir and Meyer (2010), in which Max-Q was found to be a good host to *M. arenaria* and nonhost to *M. incognita*. For whatever reason the *M. arenaria* isolate used in these studies, which was the same one used by Nyczepir and Meyer (2010) reacted differently to Max-Q is unknown at this time.

Meloidogyne spp. tomato bioassay: The results in tomato bioassay 1 indicate that Max-Q soil effectively suppressed the resurgence of *M. floridensis* infection on tomato roots, but not in Rutgers tomato soil (known host). These results are similar to previous studies reported for *M. incognita* and *M. hapla* (Nyczepir and Meyer, 2010). However, in the repeat trial (bioassay 3), the same suppressive effect on *M. floridensis* resurgence on tomato roots planted into Max-Q soil was not observed for reasons that are unknown at this time, but may be related to greenhouse temperature fluctuations. Such a temperature-related phenomenon has been reported in Nemaguard peach rootstock which is known to be resistant to *M. incognita*. It has been reported that more root galls in Nemaguard peach roots were produced by *M. incognita* at higher soil temperatures (30^{o}C) than at lower soil temperatures (25^{o}C) (Wehunt, 1972). It is thought that the mechanism for nematode resistance in Nemaguard is compromised at the higher temperatures. Similar contradictory results in the current study for *M. incognita* resurgence were also observed in bioassay 3 as compared to what was previously reported or observed in other experiments (Nyczepir and Meyer, 2010; S. H. Thomas, NMSU, pers. com.) For some unknown reason,

Max-Q was unable to suppress *M. incognita* and *M. floridensis* reproduction in the bioassay 3 soil.

Currently work is being conducted at the USDA-ARS, Byron facility that addresses ambient air temperature differences between the two different greenhouses mentioned above as an influence on root-knot nematode suppression with Max-Q. Additionally, Max-Q seed from the original seed source used in the current study will be compared to a younger aged Max-Q seed source to determine if differences in seed age may help explain the dissimilarities observed in *M. incognita* suppression.

Once the host status of Max-Q to *M. floridensis* is determined and if the results are promising, the goal is to develop a new preplant groundcover that is comparable to preplant fumigation in managing different nematode pathogens on peach in the southeastern USA.

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TABLES

Table 1. Susceptibility of tall fescue 'Max-Q' to *Meloidogyne arenaria*, *M. floridensis*, and *M. incognita* in the greenhouse 78 days after soil infestation, Trial 1[a].

Meloidogyne spp.	Egg masses/plant[b]	Eggs/gram of root	Galls/plant
M. arenaria	0 a	0 a	<1 a
M. incognita	0 a	0 a	0 a
M. floridensis	0 a	0 a	0 a

Data means of eight replications. Means within a column for given nematode species followed by the same letter are not different ($P \leq 0.05$) according to Fisher's mean separation.
[a] Initial population of *Meloidogyne arenaria*, *Meloidogyne floridensis*, and *Meloidogyne incognita* = 200 eggs/ 100 cm^3 soil.
[b] A maximum of 101 egg masses or galls were counted per plant.

Table 2. Susceptibility of tall fescue 'Max-Q' to *Meloidogyne arenaria*, *M. floridensis*, and *M. incognita* in the greenhouse 75 days after soil infestation, Trial 2[a].

Meloidogyne spp.	Egg masses/plant[b]	Eggs/gram of root	Galls/plant
M. arenaria	0 a	0 a	0 a
M. incognita	1 a	51 a	1 a
M. floridensis	2 a	129 a	7 a

Data means of eight replications. Means within a column for given nematode species followed by the same letter are not different ($P \leq 0.05$) according to Fisher's mean separation.
[a] Initial population of *Meloidogyne arenaria*, *Meloidogyne floridensis*, and *Meloidogyne incognita* = 200 eggs/ 100 cm^3 soil.
[b] A maximum of 101 egg masses or galls were counted per plant.

Table 3. Susceptibility of tall fescue 'Max-Q' to *Meloidogyne arenaria*, *M. floridensis*, and *M. incognita* in the greenhouse 74 days after soil infestation, Trial 3[a].

Meloidogyne spp.	Egg masses/plant[b]	Eggs/gram of root	Galls/plant
M. arenaria	0 a	0 a	0 a
M. incognita	4 a	853 a	14 a
M. floridensis	2 a	1477 a	4 a

Data means of eight replications. Means within a column for given nematode species followed by the same letter are not different ($P \leq 0.05$) according to Fisher's mean separation.
[a] Initial population of *Meloidogyne arenaria*, *Meloidogyne floridensis*, and *Meloidogyne incognita* = 200 eggs/ 100 cm^3 soil.
[b] A maximum of 101 egg masses or galls were counted per plant.

Table 4. Resurgence in Meloidogyne *arenaria*, *M. floridensis*, and *M. incognita* population density on tomato in soil previously planted to Max-Q in the greenhouse after 75 days, Trial 3.

Meloidogyne spp.	Egg masses/plant[a]	Eggs/gram of root	Galls/plant
M. arenaria	4 b	591 b	7 b
M. incognita	79 a	151629 a	85 a
M. floridensis	82 a	62674 a	86 a

Data means of eight replications. Means within a column for given nematode species followed by the same letter are not different ($P \leq 0.05$) according to Fisher's mean separation.
[a] A maximum of 101 egg masses or galls were counted per plant.

CHAPTER 6

USE OF SPIROTETRAMAT IN POST-PLANT MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON EGGPLANT

Shirley, A.M., Nyczepir, A.P., Noe, J.P., and P.M. Brannen. To be submitted to *Journal of Nematology*.

Abstract: Historically peach production and IPM management of nematodes has relied almost solely on pre- and post-plant applications of nematicides in the southeastern United States. Currently Telone II is the primary preplant fumigant used by peach growers, since methyl bromide and fenamiphos, the only post-plant nematicide, are no longer available. There has recently been an interest in the development of post-plant nematicides. Movento (spirotetramat; a synthetic tetramic acid, Bayer CropScience) has shown some promising nematicidal effects and is currently being evaluated on peach in the Southeast. Movento is currently registered as a broad-spectrum insecticide on peach and is classified as a Group 23 lipid biosynthesis inhibitor. Two studies using Movento were conducted from 2011-2012 with *Meloidogyne incognita* infected eggplant using various rates of spirotetramat. This study with eggplant cv. 'BlackBeauty' was performed in an attempt to establish efficacious rates for the peach studies. The study consisted of three treatments: i) Movento (0.63 kg ai/h), ii) adjuvant control, and iii) a nematode control. Each treatment was replicated six times in a randomized complete block design. All plants were inoculated with 20,000 *M. incognita* eggs and treatments were applied 10 days later. Soil samples were collected 40 and 70 days after inoculation (DAI). At 70 DAI, number of nematode eggs and dry shoot and root weights were determined. At 40 DAI Movento was effective ($P \leq 0.05$) in suppressing *M incognita* numbers compared to the controls, for both trials. At 70 DAI this effect was diminished with no differences among treatments, for both trials.

Key words: Eggplant, management, *Meloidogyne incognita*, root-knot nematode, spirotetramat

Peach production in the USA is estimated at around $600 million, with production increasing in value by $100 million in the past 10 years. In 2011, the state of Georgia currently valued peach production at $31.5 million. Though the value of peach production has increased in the past 10 years in the USA, the amount of bearing hectares planted has decreased from 59,000 hectares (145,000 acres) planted in 2002 to less than 46,000 hectares (115,000 acres) planted in 2012. In the state of Georgia the bearing hectares has decreased from 4,400 hectares (10,900 acres) in 2002 to less than 4,000 hectares (10,000 acres) in 2012 (USDA Agricultural Statistics 2012). Much of this is due to disease development within the orchard, including nematode associated diseases like Peach Tree Short Life (PTSL) and peach tree decline.

Nematode losses in peach can often be overlooked and go undiagnosed for many years. In general nematode damage is variable within an orchard. Often tree losses are gradual and increase over time with a rise in nematode population (Nyczepir, 2011). Many times this trend will continue without nematode management until a large concentration of trees are lost or production is greatly reduced within a given area. In Georgia, nematode losses are rather sporadic and lower than other states. Most growers in Georgia plant new orchards on sites with no previous history of peach to avoid having to use a preplant fumigation, due to the high costs, and to avoid any soil-borne diseases that may have build up over time, i.e. *Pseudomonas syringae pv. syringae* (Nyczepir et al., 2004). Commonly the producer puts no thought into nematode control and when losses do occur they are generally quite severe with a total loss in a given area. In South Carolina most of the nematode-associated tree losses are due to PTSL (Bertrand, 1994). In South Carolina, an average of 143,000 trees is lost to disease with a loss of around $11 million of income each year. Of this it is estimated around $6 million is lost per year to PTSL (Miller, 1994). Peach orchards have been known to survive as long as 25 to 30 years in

parts of the Southeast, though many peach trees are lost to PTSL in the first 3-5 years of planting. A successful orchard is one which will survive longer than 10 years before having to be removed (Ritchie and Clayton, 1981).

Peach tree decline, unlike PTSL, is associated with the root-knot nematode and the root-lesion nematode (*Pratylenchus vulnus*) (Nyczepir, 2011; Ritchie and Clayton, 1981). The root-knot nematodes are by far the most damaging and prevalent plant-parasitic nematodes in the world and are found in all agricultural production areas ranging from temperate to tropical climates (Lamberti, 1979; Sasser, 1979; Sasser and Freckman, 1987). The root-knot nematodes, *M. incognita* and *M. javanica* were found in 95% and 5% of peach orchards surveyed in South Carolina, respectively (Nyczepir et al. 1997).

As a plant-parasitic nematode on peach, the root-knot nematode's aboveground symptoms include: a reduction in fruit yield, plant growth, and promotion of early defoliation in severely stunted plants. Belowground symptoms include reduced root systems with malformation and galling present. Under severe conditions these symptoms can even lead to tree death (Nyczepir et al. 1993). Since the root-knot nematode is an obligate sedentary endoparasitic nematode the use of post-plant nematicides should offer measurable control.

Movento was developed and released by Bayer Crop Science and is marketed as a broad spectrum systemic insecticide. Spirotetramat is a tetramic acid which is naturally derived from fungi and other organisms. The compound has a very low level of mammalian toxicity (>5000 mg a.i. /kg bw) (Movento label, BayerCropScience). It is transported through both the phloem and xylem (ambimobile) and once inside the leaf it is hydrolyzed to its –enol, and is then moved through the phloem and xylem to both leaf and root apical meristems. It is a Group 23 lipid biosynthesis inhibitor that acts on the fecundity (number of eggs) and fertility (viable eggs) when

ingested by the organism. It has also been observed in aphids to affect edysis, leading to the incomplete shedding of the cuticle during molting. Soil activity is very short-lived with approximately 90% dissipation in one to four days; it however is active within the plant residually for two or more weeks (Bruck et al. 2009; Smiley et al. 2011, 2012; McKenry et al. 2009, 2010; Zasada et al. 2012). McKenry et al. (2009) applied spirotetramat at <100 ml/ha to *Vitis* spp, *Citrus* spp, and *Juglans* spp and observed a reduction in population of *Xiphinema* spp and *Mesocriconema xenoplax* at 36 and 56 days after treatment, respectively. A 50% population reduction was observed for three months for all plant-parasitic nematodes sampled if irrigation was withheld for up to two weeks. Sampling involving *Meloidogyne* spp, included with other species, also showed a 50% population reduction for three months. In a separate study, the effect of spirotetramat on *P. vulnus* populations in *Juglans* spp roots was evaluated for six months. A 50% *P. vulnus* population reduction was observed when applied at a rate of 441ml/ha with an adjuvant (McKenry et al. 2010). Smiley et al. (2011) applied spirotetramat at 88g/ha to two wheat fields, one in Idaho and the other in Washington, infested with the cyst nematode, *Heterodera avenae*. Results indicate that spirotetramat reduced *H. avenae* population densities by 35% and 78% in the Washington and Idaho field trials, respectively. Movento looks to be a promising post-plant nematicide for the control of plant-parasitic nematodes on peach.

MATERIALS AND METHODS

Nematode source and inoculum: The population of *M. incognita* was originally isolated from peach in Georgia, and maintained on eggplant (*Solanum melongena* cv. 'Black Beauty') in the greenhouse at the University of Georgia, Athens, respectively. Eggplant roots were processed for *M. incognita* egg inoculum using a 10% NaOCl solution (Hussey and Barker, 1973).

Root-knot nematode spirotetramat studies: Eggplant seedlings were transplanted into 15-cm-diameter standard clay pots containing 1.5 liters of a sterilized loamy sand soil mixture of 25% field soil, 50% sand, and 25% Fafard® germinating mix and placed on benches in a greenhouse (~ 27 ± 5° C). After transplanting a ~1 ml (1/4 teaspoon) of 13-13-13 was applied to each pot and water was applied as needed. Each plant was allowed to establish for one week before inoculation. Each plant, except for the water control, was inoculated with 20,000 *M. incognita* eggs/3.4 L soil. After 10 days chemical treatments were applied. The Movento study treatments included: i) Movento (0.63 kg ai/ha) ii) nematode control, and iii) adjuvant control. Movento, mixed with MES-100 adjuvant, was foliar applied to each plant at both treatment rates as recommended from the Movento label. The adjuvant control was foliar applied at 2.6 ml/L. Each treatment was replicated six times in a randomized complete block design. Soil samples were collected ~ 40 and 70 days after inoculation (DAI). At 40 DAI the soil was assayed using four soil cores (2.5-cm- diam × 15-cm-deep) and combined into one sample. Number of infective-stage juveniles (J2) were counted following extraction from a 100 cm^3 subsample of soil using soil sieves and the centrifugal-flotation technique (Jenkins, 1964). Roots from the subsample were processed for *M. incognita* eggs using a 10% NaOCl solution (Hussey and Barker, 1973). The number of J2 and eggs extracted was combined, quantified, and analyzed. At 70 DAI the remaining eggplant seedlings were taken down. A 100 cm^3 soil sample was collected for the presence of J2 nematodes and then the total population density of J2 nematodes was calculated. Foliage and shoots were removed and placed into paper bags and dry weights recorded. The roots were washed free of soil and saved for egg extraction and dry root weights. After processing the samples for J2's and eggs; the foliage, shoots, and roots were placed in an

oven dryer at 70 ºC and dry weights collected three to four days later. Samples were collected 47 DAI and 77 DAI for the first trial and 41 DAI and 83 DAI for the second trial.

Statistical analysis: Nematode and egg counts for each treatment were transformed using $\log_{10}$, analyzed using one-way ANOVA, and means separated using Fisher's combined probability test. For all studies a two-way ANOVA analysis was conducted to evaluate interactions between trials, if no significant interaction was apparent data was combined and analyzed together.

RESULTS

Since the interaction between trials within a respective study was not significant, data were combined unless otherwise stated.

Root-knot nematode spirotetramat studies: Spirotetramat was effective ($P \leq 0.05$) in suppressing *M. incognita* soil population density compared to the nematode control at 40 DAI but not the total reproduction rate (soil + roots) at 70 DAI. However, total RKN/ gram of dry root ($P \leq 0.05$) were greater ($P \leq 0.05$) in the nematode control than the spirotetramat treatment at 70 DAI (Table 1). The adjuvant control was analogous to the nematode control with no distinction between *M. incognita* populations sampled (Table 1). No plant growth differences were observed among treatments for both trials.

DISCUSSION

Root-knot nematode spirotetramat studies: Spirotetramat was effective in suppressing the first generation of *M. incognita* in eggplant at the highest labeled rate of (0.63 kg ai/ha) at the first sampling and also had significantly lower eggs/ gram of dry root at the second sampling. The reason spirotetramat was unable to control total RKN populations at both sampling dates is unknown. Given these promising results, it has become apparent the possible benefits of

spirotetramat for use of nematode control on peach. So, this study will be repeated with both *Meloidogyne incognita* and *Mesocriconema xenoplax* on peach in the greenhouse.

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TABLES

Table 1. Effect of spirotetramat on *Meloidogyne incognita* reproduction on 'BlackBeauty' eggplant in the greenhouse 40 and 70 days after inoculation in Athens, Georgia

	RKN/ 100cm^3 soil[a]	RKN/pot[b]	RKN/g dry root system[c]
Treatment[d]	40 DAI	70 DAI	70 DAI
NC	60381 a[e]	1059340 a	190398 a
ADJ	46302 a	1069447 a	201134 a
SPT	5578 b	730920 a	105744 b

Data are means of 12 replications (two studies data combined).
[a] RKN/100 cm^3 soil = number of *M. incognita* J2 per 100 cm^3 soil combined with number of eggs extracted from root segments obtained from 100 cm^3 soil subsample.
[b] Total number of J2 and number of eggs per root system.
[c] Total RKN per plant divided by total dry root weight.
[d] NC = nematode control, ADJ = adjuvant, and SPT = spirotetramat (0.63 kg ai/ha).
[e] Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Fisher's combined probability test.