

DELIVERY, MOVEMENT, AND EFFICACY OF NEMATICIDES FOR MANAGEMENT OF PLANT-PARASITIC NEMATODES IN VEGETABLES

by

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ABSTRACT

Vegetable production in plasticulture system is common in Georgia and Southeastern United States. Root-knot nematodes (RKN) are one of the serious pests of vegetable crops and can drastically reduce yield if not effectively managed. This study investigated the best chemical options for RKN management in a double-cropped vegetable plasticulture system. We found Pic60, a mixture of chloropicrin (59.6%) and 1,3-dichloropropene (39%), to be a good choice for effective RKN control and yield increase in pepper grown on plastic beds in the spring season. Also, non-fumigant nematicides (fluensulfone, fluazaindolizine, fluopyram, oxamyl, *Burkholderia* spp. strain A396) were applied in the same plastic beds in the summer season and reduced RKN damage in squash. In another study seeking to optimize the drip application of non-fumigant nematicides for RKN control, we observed that the surface and sub-surface application of fluensulfone, fluazaindolizine, and fluopyram had comparable effects to the surface application of the nematicides.

INDEX WORDS: Root-knot nematode, plasticulture, fumigant, non-fumigant nematicide,
double-crop vegetable, sub-surface drip tape

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DEDICATION

This work is dedicated to me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background and vegetable production in Georgia

Georgia is ranked among the leading vegetable producing states (USDA-NASS, 2018); the total vegetable crops produced in 2019 had a farm gate value of \$1.18 billion (Kane, 2021) and ranked among the top four commodities with the highest farm gate value in Georgia (Stubbs, 2020). Vegetable production is done year-round (Westerfield and Linvill, 2015) with counties in the southern region accounting for 64% of the \$1.18 billion total farm gate value. These counties include Echols, Colquitt, Tift, Crisp, Mitchell, Decatur, Worth, and Brooks. The top ten valued vegetable crops grown in Georgia are watermelon, sweet corn, onion, bell pepper, cucumber, cabbage, carrot, tomato, yellow squash, and eggplant (Stubbs, 2020). Growing vegetable crops in plasticulture is a common practice in Southeastern United States including Georgia. The plasticulture system incorporates plastic mulch, raised beds, drip irrigation and fumigation in the production of crops (Lamont, 2004a; Sanders et al., 1996). Its use in commercial vegetable production dates back to the early 1960s (Lamont, 2004a) and it offers several benefits including the reduction of fertilizer leaching by preventing rainfall percolation through the soil, increased soil temperature to allow early crop production and improved fruit quality by preventing contact with the soil and potential soil-borne pathogens that cause fruit rot. The plasticulture system also reduces soil compaction, allows for better fumigation and improves surface water drainage (Lamont, 2004a; Sanders et al., 1996). Laying of the plastic mulch is usually done with a plastic

mulch laying machine that may be attached to a fumigation rig. The plastic mulch laying machine makes raised beds, lays the plastic over it, and inserts drip tape underneath the plastic mulch; formation of raised beds and plastic mulch laying can also be done in separate operations using different equipment (Lamont, 2004b; Sanders et al., 1996).

Despite the numerous advantages the plasticulture system offers, the cost for initial setup and removal and disposal after the cropping season are two major challenges (Sanders et al., 1996). To limit this burden and increase economic returns, growers usually use a single plastic mulch application for two to three cropping seasons. An example of this cropping practice would be to lay the plastic mulch in the spring and plant tomato or pepper, and reuse the same plastic mulch in growing summer squash, cucumber, or cole crops in the fall (Lamont, 2004a; Sanders et al., 1996). This double- or triple cropping practice on plasticulture encourages the buildup of population of plant-parasitic nematodes (PPN), in particular root-knot nematode (RKN; *Meloidogyne* spp.).

Root-knot nematode, *Meloidogyne* spp.

The genus *Meloidogyne* is associated with the production of vegetable crops worldwide (Hallmann and Meressa, 2018) and contains over 100 described species (Karssen et al., 2013). A recent survey of 436 vegetable fields conducted in southern Georgia, USA in 2018 reported the RKNs to have an incidence rate of 67%, the highest among all PPNs detected in the study (Marquez et al., 2021). Some species of RKN that inflict damage on vegetable crops include *M. arenaria*, *M. javanica*, *M. incognita*, *M. hapla*, *M. haplanaria*, *M. chitwood*, *M. enterolobii* (syn. *M. mayaguensis*), and *M. floridensis* (Brito et al., 2008; Hallmann and Meressa, 2018; Joseph et al., 2016; Moens et al., 2009). Stunted growth, poor development, early die-back, slow maturity,

yield loss and compromised quality of marketable produces are characteristics of vegetable crops infected by RKN (Hallmann and Meressa, 2018).

During host colonization and infection, the RKN second-stage juvenile (J2) penetrates the root and initiates the formation of feeding sites called giant cells. The giant cells are multinucleate, hypertrophied feeding cells originating from parenchymatic root cells. After giant cell initiation, the nematode molts to third-stage juvenile, fourth-stage juvenile and finally to the adult stage. The adult female is pear-shaped and exudes egg from its posterior end into gelatinous egg masses that lies outside the galled root tissue. The first-stage juvenile develops inside the egg, but it hatches from egg as infective J2, and the parasitic life cycle begins. Adult male RKN are vermiform in shape and rarely occur. The primary symptom of plants infected with RKNs is galling of the root system. There are however certain species such as *M. artiellia* that do not cause root galling (Hallmann and Meressa, 2018; Moens et al., 2009). The type of species, host plant, and temperature influence the duration of RKN life cycle. For example, species of RKN prevalent in warmer regions such as *M. incognita* and *M. javanica* thrive well in the temperature range of 25-30 °C, and species that occur in cooler regions such as *M. hapla* do better in temperature range of 18-25°C (Hallmann and Meressa, 2018).

RKN also forms a disease complex with other plant pathogens, in particular fungal pathogens, and this can exacerbate the plant condition (Manzanilla-López and Starr, 2009). Sumner and Johnson (1973) showed that *M. incognita* increased the susceptibility of watermelon cultivars to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum*. Hasan (1985) reported that *Rhizoctonia solani* and *Pythium aphanidermatum* was involved in the increased susceptibility of two nematode-resistant cultivars of chili peppers to *M. incognita*. In a growth chamber experiment, Morris et al. (2016) demonstrated that cucumber plants inoculated with

both *M. incognita* and *Pythium aphanidermatum* had a higher incidence of damping-off than the non-inoculated plants or plants inoculated with only one of the two pathogens.

Other PPNs associated with vegetable crops are the stubby-root [*Nanidorus* spp. (syn. *Paratrichodorus* spp.) and *Trichodorus* spp.], reniform (*Rotylenchulus reniformis*), false root-knot (*Nacobbus aberrans*), cyst (*Heterodera* spp. and *Globodera* spp.), root-lesion (*Pratylenchus* spp.), stem and bulb (*Ditylenchus* spp.), sting (*Belonolaimus longicaudatus*), burrowing (*Radopholus similis*), stunt (*Tylenchorhynchus brassicae*), dagger (*Xiphinema* spp.), and awl (*Dolichodorus* spp.) nematodes (Crow, 2020; Crow, 2018; EPPO, 2009; Hafez and Palanisamy, 2016; Hallmann and Meressa 2018; Hajihassani et al., 2018b; Harveson, 2008; Wang, 2016).

Nematode management approaches and justification for this research

There exist several means for RKN management in vegetable cropping systems, and these management options can be broadly classified into chemical, cultural, biological and host plant resistance. The chemical option includes the use of fumigant and non-fumigant nematicides. Cultural control includes crop and fallow rotation, solarization and steam heat, flooding, sanitation, and use of trap crops, cover crops, and soil amendments. Biological control involves the use of nematode antagonistic micro-organisms whereas host plant resistance involves growing crops with genes that reduces and, in some instance, eliminates their susceptibility to RKNs (Crow, 2020; Moens et al., 2009; Noling, 2006). The plasticulture practice of double- or triple cropping on a single application of plastic mulch, however limits management choices for nematode disease control. For example, crop rotation and fallow are not economically viable since more than one cropping season is done on a single application of plastic mulch before removal. Host resistance seems to be a good option for RKN control and resistant genes have been identified in multiple vegetable crops. For example, the *Mi* gene of

tomato confers resistance to *M. arenaria*, *M. incognita* and *M. javanica*. The gene was first identified in a wild tomato species (*Lycopersicon peruvianum*) and later introgressed in cultivated tomato (*L. esculentum* Mill., now known as *Solanum lycopersicum* Linnaeus) (Williamson, 1998). Rich and Olson (1999) noted that tomato cultivars with the *Mi* gene had much lower root galling than susceptible cultivars. In pepper, the *N* and *Me* genes confer resistance to some species of RKN (Hare 1956; Fery and Dukes 1996; Djian-Caporalino et al. 2001; 2007). Hajihassani et al. (2019b) reported that RKN-resistant bell pepper cultivars/lines (Charleston Belle, HDA-149, HDA-330, PM-217, PM-687) carrying the *N*, *Me1* and *Me3* genes reduced the penetration and reproduction of *M. incognita* race 3, *M. arenaria* race 1, *M. javanica*, and *M. haplanaria*. Thies et al. (2008) demonstrated that two RKN-resistant bell pepper cultivars (Charleston Belle and Carolina Wonder) with the *N* gene greatly suppressed root galling caused by *M. incognita* than their susceptible parents (Keystone Resistant Giant and Yolo Wonder B).

Some shortcomings have however been associated with nematode-resistant varieties. There have been reports of resistance breakdown at high soil temperatures (Dropkin, 1969; Thies and Fery, 2002) and by virulent or resistance-breaking populations of nematodes (Castagnon-Serono et al., 1996; Kaloshian et al., 1996; Ornat et al., 2001; Tzortzakakis et al., 2005). There are also few nematode-resistant varieties commercially available to growers (Hajihassani, 2018a), and poor agronomic traits such as yield have been associated with some resistant varieties (Nnamdi et al., 2022).

Biological control is also a means to manage RKN in the plasticulture system. Bacteria and fungi that are antagonistic to RKN are generally utilized in this management method (Forghani and Hajihassani, 2020). Some efficacious bacteria against the RKN are *Bacillus*

thuringiensis, *Bacillus firmus*, *Burkholderia cepacia*, *Burkholderia rinojensis*, and *Pasteuria penetrans* (Forghani and Hajihassani, 2020; Santos et al., 2016; Timper, 2011). *Arthrobotrys* spp., *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Trichoderma harzianum* and *T. koningii* are some species of fungi that have been documented to be effective against the RKNs (Duponnois et al., 1996; Kiewnick and Sikora, 2006a; Van Damme et al., 2005; Windham et al., 1989). Some nematode antagonistic bacteria and fungi have been developed commercially and formulated into products that can be applied by chemigation, soil drenching, foliar spray, broadcasting, or in-furrow to the soil for RKN control. Example of such products are Majestene® formulated from heat-killed cells of the bacterium *Burkholderia rinojensis* strain A396 (Santos et al., 2016), MeloCon® WG and BIOACT® WG from *Paecilomyces lilacinus* strain 251 (Greenbook, 2021; Kiewnick and Sikora, 2006b), and Flocter® from *Bacillus firmus* I-1582 (Hitzberger et al., 2014). Several studies have been conducted showing the efficacy of these bio-nematicides products (Khanal and Desaegeer, 2020; Kiewnick and Sikora, 2006a; 2006b; Santos et al., 2016); however, there have been reports of less efficacy compared to chemical nematicides (Raddy, Fouad and Montasser, 2013) in fields with high nematode population densities (Van Damme et al., 2005).

Currently, chemical control seems to be a better option for RKN management in vegetables planted in the plasticulture system. Growers usually apply a fumigant chemical product while laying the plastic mulch for the first crop because of their high efficacy and broad-spectrum effect on other pests/pathogens (Morris et al., 2015; Sanders et al., 1996). Soil fumigation effectively controls the RKNs in the first crop, but the efficacy of chemical's active ingredients wears off afterward and offers little control to the population of RKNs in the second crop grown (Giannakou et al., 2002). Therefore, drip-applied nematicides are used in lieu of the

chisel-injected fumigants to control the nematodes in the second crop because application of the chisel-injected fumigants will destroy the plastic mulch (Morris et al., 2015). Drip-applied nematicides include non-fumigant nematicides and fumigants formulated as an emulsifiable concentrate (Desaeger and Watson, 2019; Desaeger et al., 2008). Chemical control being a better management option for nematodes pests' control of vegetables in plasticulture system necessitates research to seek best chemical choices and practice.

Chemical control. Nematicides can be broadly classified into two groups based on their volatility in soil: fumigants and non-fumigant nematicides. Fumigants are highly volatile chemical compounds that are mostly characterized by broad-spectrum activity and have a multi-site inhibition mode of action (MOA) (Desaeger et al., 2020; Noling, 2008; Sparks et al. 2020). They also have a long plant-back date to prevent phytotoxicity damage, and their application usually requires specialized equipment, safety covering and an applicator license (Noling, 2008). Fumigants usually have a high toxicological profile on humans and the environment. The signal word for products in this class is “danger” which makes proper handling, safety measures, and buffer zones important during application (Desaeger et al., 2020; Noling, 2008).

Methyl bromide was the dominant fumigant for decades with over 27,000 metric tons used annually. This was due to its high efficacy against several pathogens and pests; however, the discovery of its negative effect on the stratospheric ozone layer led to a reduction in its production from 1999 and an eventual phaseout in 2005. The current exception for the use of methyl bromide is only based on critical use exemptions, emergencies, quarantine and pre-shipment applications (Johnson et al., 2012; Ragsdale and Vick 2001; US EPA 2020). Ever since the ban of methyl bromide, several alternative fumigants have been sought after and research has been conducted on the efficacy of these alternatives in controlling RKN in vegetable crops

(Desaeger and Csinos, 2006; Desaeger et al., 2008; Desaeger et al., 2017; Giannakou et al., 2002; Gilreath et al., 2004). Some fumigants available to growers for RKN control in vegetables are 1,3-dichloropropene, chloropicrin, dimethyl disulfide, allyl isothiocyanate, metam sodium and metam potassium (Culpepper et al., 2017; Dittmar et al., 2020; Hajihassani, 2018a; Kemble et al., 2021).

1, 3-dichloropropene (1, 3-D) is a clear, colorless liquid, strong irritant, and used as a soil fumigant (NCBI, 2021a). Reports on its nematicidal properties date as far back as the 1940's (Carter, 1943). Several other studies have also affirmed the ability of 1, 3-D to control PPNs and increase the yield and vigor of vegetable crops (Desaeger et al., 2017; Minnis et al., 2004; Qiao et al., 2010). Chloropicrin is another fumigant considered as an alternative to methyl bromide. It is a C-nitro compound, slightly oily colorless to yellow liquid, and has a strong irritating odor (NCBI, 2021b). Gilreath et al. (2004) reported that Chloropicrin reduced the RKN gall incidence on cucumber in both fall and spring trials; they also reported that Chloropicrin decreased the purple nutsedge population density in a bell pepper field by 91% and 96% six weeks after transplanting during the spring and fall trials, respectively. Dimethyl-disulfide, a recently registered fumigant, has a zero-ozone depletion potential and elicits its pesticide effects by causing a dysfunction of the mitochondria, activation of ATP sensitive potassium channels, and inhibition of the cytochrome oxidase (Auger and Charles, 2003; Charles, 2003). Using both greenhouse and field trials, Fritsch et al. (2014) demonstrated that dimethyl-disulfide was effective against *Meloidogyne* sp., *Pratylenchus* sp., *Heterodera carotae* and *Globodera pallida* of vegetable crops. Also, Zanón et al. (2014) reported the nematicidal efficacy of dimethyl-disulfide against RKN populations in pepper and cucumber crops and a significant yield increase. Allyl isothiocyanate is a soil biofumigant and occurs naturally in mustard oil (Isagro

USA, 2021). It has been shown to control soilborne diseases, *Meloidogyne* spp., and weeds (Ren et al, 2018).

Non-fumigants nematicides are nonvolatile chemicals with a narrow spectrum of activity. Unlike fumigants, they do not need specialized equipment for application and can be applied by soil drench, drip irrigation, broadcast, in-furrow, and foliar spray. Some non-fumigants nematicides available to growers for RKN control in vegetables are oxamyl, fluensulfone, fluopyram, ethoprop, spirotetramat, terbufos and fluazaindolizine (Hajihassani, 2018a). Older non-fumigants such as oxamyl, ethoprop and terbufos which belong to the organophosphates and carbamates chemical subgroup have the signal word of “danger” on their product label (Desaeger et al., 2020). The mode of action of organophosphates and carbamates on nematodes is by inhibiting acetylcholinesterase production (Desaeger et al., 2020; Sparks et al., 2020). Newer non-fumigant nematicides such as fluensulfone, fluopyram, spirotetramat and fluazaindolizine have the signal word of “caution” on their product label (Desaeger et al., 2020). The product label of caution eliminates the need for a pesticide applicator license for handling and use.

The nematicidal efficacy of the non-fumigant nematicides has been documented extensively. Oxamyl is one of the older non-fumigant nematicides belonging to the family of pesticides known as carbamates (Hayes, 1982; NCBI, 2021c). Reports on its nematicidal activity date as far back as the 1970s and its efficacy against insects has also been shown (Abawi and Mai, 1972; Kerns and Tellez, 1998; Kinloch, 1972). A recent microplot study on cucumber however observed that oxamyl had less nematicidal efficacy when the soil population of *M. incognita* was above 5,000 juveniles suggesting that it might be less effective in RKN control than newly introduced non-fumigant nematicides in soils with high infestation pressures (Hajihassani et al., 2019a). Fluensulfone is a member of the heterocyclic fluoroalkenyl sulfone

chemical subgroup, and its MOA is unknown (Desaeger et al., 2020; Sparks et al., 2020). Morris et al. (2015) evaluated the efficacy of fluensulfone in the management of *Meloidogyne* spp. in a tomato-cucumber double-cropping system and noted that it reduced the root gall severity of tomato by 73%. Fluensulfone has also been reported to have systemic activity against *M. incognita* on peppers (Oka et al., 2012). Fluopyram belongs to the pyridinyl-methyl benzamides chemical subgroup, and its MOA is the inhibition of mitochondrion electron transport (Desaeger et al., 2020; Sparks et al., 2020). It also has fungicidal properties (Avenot and Michailides, 2010) and was initially registered as a fungicide before discovering its nematocidal activity later on. Faske and Hurd (2015) reported the nematostatic effect of fluopyram against *M. incognita* and *R. reniformis* in tomato even when applied at a low concentration of 1.0 µg/ml. Also, fluopyram reduced the RKN population of lima bean and increased yield during experimental trials in the greenhouse and microplot conditions (Jones et al., 2017). Fluazaindolizine is a selective nematicide currently undergoing final registration and regulatory procedure before introduction to the pesticide market. It is tagged to be selective in action because a bioassay study showed fluazaindolizine to be efficacious against RKN while having no adverse effect on a bacterivorous nematode (*Acrobeloides buetschlii*) (Thoden and Wiles, 2019). Fluazaindolizine belongs to the imidazopyridine chemical subgroup, and its MOA is unknown (Desaeger et al., 2020; Sparks et al., 2020). Nunez (2017) reported that fluazaindolizine decreased root-knot nematode injury on tomato by 69% compared to the control treatments.

Sorption and mobility of fluazaindolizine

Sorption is a physicochemical process whereby a sorbate is attached to a sorbent (Kousksou et al., 2014). It is a process that influences the interaction between pesticide molecules and the solid phase in the soil. This interaction is facilitated by specific mechanisms

such as ionic and covalent bonds, hydrogen bonding, van der Waals forces, and hydrophobic interactions (Ahmad, 1999; Khalid et al., 2020; Khan, 1980). Sorption governs pesticide efficacy and their fate in the soil, and it is controlled by two major factors: i) the properties of the pesticides and ii) the properties of the soil (Bailey and White, 1970; Khan, 1980). Pesticidal properties that influence sorption are their solubility in water, ionizability and nature of the pesticide formulation. For example, sorption generally increases for pesticides with the following ionizable functional groups: $-R_3N^+$, $-CONH_2$, $-OH$, $-NHCOR$, $-NH_2$, $-OCOR$, and $-NHR$ (Ahmad, 1999). Soil properties that affect sorption include the soil organic matter, clay content, pH, water content, temperature, and presence of organic cosolvents in soil solution. Among the soil properties, prominent ones are the soil organic matter and clay content. Soils high in organic matter usually have a higher sorption activity (Ahmad, 1999). Also, soils with a 2:1 type of clay minerals generally have a greater capacity for sorption than soils with a 1:1 type of clay minerals (Bailey and White, 1970). The soil properties also play a role in the nematicide activity against the target nematode pest and mobility in the soil. Oka et al. (2013) reported that the efficacy of fluensulfone against *M. javanica* and mobility in the soil were reduced by the addition of peat (organic matter). They also noted that clay restricted the movement of fluensulfone in the soil. Noling (2011) stated that there is less downward movement of nematicide and more dispersion in heavier clay soils composed of smaller pore spaces and aggregated compact structures than sandy soils consisting of less aggregated soils and large pore spaces.

Aside sorption, other prominent processes that play a role in pesticide activity and their behavior in the soil are volatilization (Glotfelty and Schomburg, 1989), chemical transformation (Kookana et al., 1997), photolysis (Burkhard and Guth, 1979; Kromer et al., 2004), microbial degradation (Karpouzias et al., 2004; Meher et al., 2010), and leaching (Bilkert and Rao, 1985;

Karpouzas et al., 2007). The sorption and mobility of several nematicides in different types of soils have been investigated. Morris et al. (2018) noted the Freundlich adsorption capacity (Kf) of fluensulfone to be 1.24 – 3.28 mL/g and also reported the nematicide to be relatively mobile in the soil. Zhou et al. (2021) documented the Kf for fluopyram to be 5.52 – 6.80 mL/g and reported that most of the nematicide was distributed in the top 0 – 10 cm of the soil. Faske and Brown (2019) also demonstrated the nematicide to have high mortality of *M. incognita* at a depth of 0 – 10 cm in sandy soil and 0 – 5 cm in sandy loam soil. There exists little information on the sorption and soil mobility of fluazaindolizine.

Several *in vitro* experiments have been conducted to evaluate the effect of nematicides on RKN (Faske and Hurde, 2015; Oka and Saroya, 2019; Shirley et al., 2019). For fluazaindolizine, Thoden and Wiles (2019) observed the nematicide to cause up to 99% mortality of RKN after an exposure period of 168 hours at 250 ppm and 30 °C. Thoden et al. (2019) also showed fluaziandolizine to cause mortality of 39–77% at 50 ppm after a 72-hours incubation period in the nematicide. These studies were however done on Petri-dishes. No *in vitro* work on the effect of fluazaindolizine on RKN has been done that considers the soil environment's impact on the nematicide activity.

Objectives

The overall goal of this research is to add another chemical management tool and/or technique to the toolbox of the plasticulture vegetable growers. Specifically, my research objectives are:

1. Evaluate the efficacy of different fumigant and non-fumigant nematicides on RKN in a pepper-squash plasticulture system.

2. Evaluate the effects of surface and sub-surface application vs. only surface application of non-fumigant nematicides on RKN and tomato yield.
3. Compare the sorption and mobility of fluazaindolizine on different soil series from Georgia. And subsequent evaluation of the impact of fluazaindolizine movement in the soil columns on *M. incognita* control during *in vitro* sensitivity assay.

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CHAPTER 2

ROOT-KNOT NEMATODE MANAGEMENT FOR PEPPER AND SQUASH ROTATIONS
USING PLASTICULTURE SYSTEMS WITH FUMIGANTS AND NON-FUMIGANT
NEMATOCIDES

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Abstract

Multi-cropping of vegetables on the same plastic mulch builds up the population of root-knot nematodes (RKN; *Meloidogyne* spp.), which can severely reduce crop growth and yield. Vegetable growers in the southeastern US usually fumigate soil while laying the plastic mulch in the spring. They then apply non-fumigant nematicides via drip irrigation systems for subsequent crops grown on the mulch. With the advent of new and emerging nematicides, this research was aimed to investigate the best chemical control practice for *M. incognita* in a pepper and squash plasticulture system. Field trials were conducted in the spring (pepper) and summer (squash) of 2019 and 2020. The spring treatments were soil fumigants of 1,3-dichloropropene, allyl isothiocyanate, and 1,3-dichloropropene plus chloropicrin (Pic60), a RKN-resistant cultivar (Carolina Wonder), and an untreated check. Summer treatments were the non-fumigant nematicides fluopyram, fluensulfone, fluazaindolizine, oxamyl, and *Burkholderia* spp. strain A396. All spring treatments, except allyl isothiocyanate, reduced ($P < 0.05$) root galling compared to the untreated check at pepper harvest. At the end of the season, the population density of *M. incognita* in the soil was only lower ($P < 0.05$) for the RKN-resistant cultivar treatment than that of the untreated check. Though the RKN-resistant cultivar treatment had the lowest ($P < 0.05$) soil RKN population and significantly reduced root galling, it had the lowest pepper fruit yield. In contrast, pepper associated with Pic60 treatment had the highest fruit yield. For the summer trial, squash plots treated with fluensulfone had the lowest root gall index and oxamyl had the highest ($P < 0.05$); however, no difference was observed between fluensulfone and oxamyl with other treatments. In 2019, plots treated with *Burkholderia* spp. and fluensulfone had higher fruit yield ($P < 0.05$) than fluazaindolizine; however, squash yield was similar among the treatments in 2020. This study suggests that Pic60 (a mix of nematicide and fungicide active

ingredients) is likely an ideal fumigant to apply when laying the plastic mulch because of its broad-spectrum effect, and any of the non-fumigant nematicides may be used in RKN control on squash.

Introduction

Georgia is ranked among the leading vegetable producing states in the United States (USDA-NASS, 2018). The total vegetable crops produced in 2019 had a farm gate value of \$1.18 billion (Kane, 2021) and ranked among the top four commodities with the highest farm gate value in Georgia (Stubbs, 2020). Vegetable production is done year-round (Westerfield and Linvill, 2015) with over one-third of this on plasticulture (Hajihassani, 2018). Plasticulture is a cropping system in which crops are grown on raised beds covered with plastic mulch and fitted with drip irrigation. This cropping system boosts crop yield by offering many advantages, including enhancing temperature absorption and retaining soil moisture, fertilizers, and chemical fumigants (Sanders et al., 1996). Vegetable growers in the southeastern US widely utilize this cropping system because of its numerous benefits. Fumigation of the soil provides weed, nematode and disease control while laying the plastic mulch. Application of emulsified formulations of fumigant or non-fumigant nematicides via drip-tape irrigation system for subsequent crops grown on the mulch can further improve nematode control. A single mulch application can be used to produce different vegetable crops over multiple seasons (Desaeger and Csinos, 2006; Morris et al., 2015) to reduce the cost arising from the initial installment as well as removal and disposal of the mulch after each cropping season (Morris et al., 2015; Sanders et al., 1996). However, this practice often results in increased population density of root-knot nematodes (RKN; *Meloidogyne* spp.) that can severely reduce the crop growth and yield

(Hajihassani, 2018). *Meloidogyne* spp. cause significant damage to yields of different vegetable crops, for example; up to 60% on cucumber (Ornat et al., 1997); 80% on bell pepper (Di Vito et al., 1985) and 85% on tomatoes (Barker et al., 1976). Marquez et al. (2021) reported a greater abundance of *Meloidogyne* spp. in soil under plastic beds than bare ground production systems of vegetables in Southern Georgia.

Several plant-parasitic nematodes (PPN) infest and parasitize vegetable crops, but RKN is the most prevalent and damaging (Hallmann and Meressa, 2018; Hajihassani et al., 2019a; Marquez et al., 2021). A survey of 436 vegetable fields in southern Georgia in 2018 identified RKN in 67% of the fields sampled with nematode counts ranging from 1 to 14,144 second-stage juveniles per 100 cm³ of soil (Marquez et al., 2021). Damage symptoms on vegetable crops due to the RKN infestation are stunted growth, poor development, slow maturity, yield loss, and compromised quality of marketable production. More recently, resistant-breaking populations of RKNs have also been reported in vegetable producing areas in Georgia, USA (Hajihassani et al., 2021). RKN can also form disease complexes with other soilborne pathogens such as *Fusarium* or *Pythium* and further exacerbate the plant growth and increase its yield loss (Sumner and Johnson 1973; Morris et al., 2017).

Since the phase-out of methyl bromide as a fumigant and cancelation of many organophosphates and carbamates registration, alternative fumigants have been adopted along with the labeling of new nematicides (Oka, 2020). Alternative fumigants include 1,3-dichloropropene (1,3-D), metam sodium, chloropicrin (Pic), dimethyl disulfide (DMDS), and allyl isothiocyanate (AITC) (Gilreath et al., 2004; Ren et al., 2018; Roskopf et al., 2006). When fumigants are applied to the first crop in the plasticulture systems in the spring, they often do not provide residual control to the next crop. Therefore, nematicides must be applied again to protect

this subsequent crop from nematode damage (Desaeger and Watson, 2019; Giannakou et al., 2002). Fumigants are usually injected into the soil through specialized equipment two-three weeks before planting to avoid phytotoxicity damage (Noling, 2008). Some fumigants such as 1,3-D, chloropicrin and metam sodium can also be applied as an emulsifiable concentrate (EC) via the drip irrigation system for nematode control; however, their nematicidal effectiveness might be limited because of uneven distribution in the bed (Desaeger and Csinos, 2006; Desaeger et al., 2008).

Non-fumigant nematicides are an alternative for application via the drip tape because of the limiting factors of fumigants. Organophosphates, organochlorines, and carbamates were the first set of non-fumigants to be developed. However, registrations were canceled for most of them because of their adverse effects on humans and non-target organisms (Oka, 2020). Oxamyl, a carbamate product, has been commonly used by vegetable growers to control PPN (Desaeger and Csinos, 2006) but it seems to be *less efficacious* against the RKN in soils with high population density (Hajihassani et al. 2019a). Furthermore, oxamyl toxicity categorization as Danger/Poison limits it to a Restricted Use Pesticide and mandates a pesticide applicator license requirement during purchase, handling, and utilization (US EPA, 2018). New non-fumigant nematicides with reduced toxic side-effects on non-target microorganisms, insects or mammals are the fluorinated nematicides which include fluopyram, fluensulfone, and fluazaindolizine. Fluopyram was initially developed as a fungicide, and its nematicidal activity was discovered later. Fluensulfone was registered for use in 2014, and fluazaindolizine is under review (Desaeger et al. 2020; Oka, 2020). All three nematicides are efficacious in the control of RKN in different vegetable crops. Hajihassani et al. (2019a) in microplot studies involving different population densities (1,000, 5,000, 10,000, and 20,000 nematodes/microplot) of *M. incognita*

noted that fluopyram, fluensulfone and fluazaindolizine significantly reduced root galling and nematode numbers in the soil in cucumber. They also documented that fluensulfone and fluazaindolizine were the most effective nematicides for *M. incognita* control, and oxamyl was the least effective at the highest nematode densities. Also, in multi-year carrot field trials, Becker et al. (2019) reported fluopyram, fluensulfone and fluazaindolizine to be efficacious in reducing root galling caused by *M. incognita*.

Biologically-based nematicides are environmentally friendly chemistries derived from nematode antagonistic fungi or bacteria (Forghani and Hajihassani, 2020). Majestene is an example of a bionematicide that contains heat-killed *Burkholderia rinojensis* strain A396 that has been shown to reduce PPN populations in both greenhouse and field experiments (Cordova-Kreylos et al., 2015; Santos, 2017). Nematode-resistant cultivars of pepper (*Capsicum annuum* Linnaeus) are also a mean for the management of *Meloidogyne* spp. Several genes including the *N* and *Me* genes that confer resistance to *M. incognita*, *M. javanica*, *M. arenaria*, and some isolates of *M. hapla* have been characterized, and some elite genes were introgressed into pepper (Djian-Caporalino et al. 1999; Thies and Fery, 2000; Rutter et al., 2018). A field study conducted in Florida has shown significant reductions in root galling and reproduction of *M. incognita* in two commercially available cultivars of pepper, Charleston Belle and Carolina Wonder (Thies et al., 2008). Hajihassani et al. (2019b) reported that the cultivar Charleston Belle reduced the penetration and reproduction of *M. incognita* race 3, *M. arenaria* race 1, *M. javanica*, and *M. haplanaria*. Djian-Caporalino et al. (2014) reported that a hybrid pepper carrying the *Me1* and *Me3* genes was effective in reducing soil RKN populations in the long term which improved root protections of subsequent crops against the nematode infection. The use of resistant peppers as a

trap crop has also been shown to suppress the soil nematode infestation as much as 99 and 80% after the first and second implementation of the trap crop (Navarrete et al., 2016).

This research was conducted to investigate the efficacy of different fumigants when laying the plastic mulch and detect the best non-fumigant nematicide for use in the second crop in a pepper-squash rotation in a plasticulture system. A resistant cultivar of pepper was also evaluated to compare its efficacy to the fumigants in RKN control and examine whether it could be used as a rotation crop for nematode management in the double-cropped squash treated with five non-fumigant nematicides.

Materials and methods

Site description and land preparation. This study was conducted at the University of Georgia Black Shank Farm, Tifton, GA in the spring and summer of 2019. A repeat experiment was conducted on the same experimental site in the spring and summer of 2020. The soil at the experiment site is a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with pH of 5.06–5.88 and a history of infestation with different PPN including root-knot, stubby root (*Paratrichodorus* spp.), spiral (*Helicotylenchus* spp.), and ring (*Mesocriconema* spp.) nematodes. Okra (*Abelmoschus esculentus* Linnaeus) and hairy vetch (*Vicia villosa* Roth) were consecutively grown in fall and winter 2018, respectively to increase the *M. incognita* population. In winter 2019 (following the completion of trials), only hairy vetch was grown; however, since the nematode infestation pressure was low, the experiment fields were inoculated with *M. incognita*-infected roots of eggplant (*Solanum melongena* Linnaeus) produced in the greenhouse at 25-28 °C for three months. These roots were chopped up into tiny bits and scattered across the fields for a rototiller to till it into the soil at a depth of 12.7 cm. The average

number (\pm standard deviation) of *M. incognita* second-stage juvenile (J2) in the soil prior to 2019 and 2020 trials were 15 (\pm 15.2) and 2.5 (\pm 2.7) J2 per 100 cm³ of soil, respectively.

The fields were harrowed and rototilled prior to the trial establishment in each year. All pesticides and fertilizer applications were done following recommendations based on the Georgia pest management handbook and the Southeastern US vegetable crop handbook (Kemble et al., 2021). Briefly, a pre-emergence herbicide—s-metolachlor (Dual Magnum; 83.7% AI; Syngenta, Wilmington, DE) was applied at 1.2 L/ha before fumigation and laying the plastic mulch. 10-10-10 NPK granular fertilizer (Super Rainbow Plant Food; Nutrien, Loveland, CO) with the following formulation (10% N, 10% P, 10% K, 3% Ca, 2% Mg, 12% S, 0.07% B, 0.25% Mn, 0.1% Zn, 6% Cl) was incorporated into the soil at 560 kg/ha with a rototiller on 15 March and 16 March in 2019 and 2020, respectively. Drip application of 7-0-7 NPK liquid fertilizer (Big Bend Agri-Services Inc; Cairo, GA) with the following formulation (7% N, 0% P, 7% K, 2% Ca, 0.5% S, 0.05% B, 0.05% Mg, 0.05% Zn, 0.02% Mn) at 247 L/ha was first used two weeks after transplanting and continued weekly until harvest. Glyphosate (Credit 41 Extra; 41% AI; Nufarm Inc, Alsip, IL) was applied in between the rows and at the ends of the plots using a hooded sprayer at 2.3 L/ha for weed management in the pepper trials on 7 May, 24 May, and 13 June in 2019 and on 6 May and 1 June in 2020. Glyphosate was not utilized during the squash trials because weed management in between the rows and at the ends of the plots was not an issue. Penthiopyrad (Fontelis; 20.4% AI; Corteva Agriscience, Indianapolis, IN) was applied via drip irrigation for the control of southern blight in the peppers. Azoxystrobin plus difenoconazole (Quadris Top; 18.2% + 11.4% AI; Syngenta Crop Protection LLC, Greensboro, NC) was sprayed on the squash plants for the control of powdery mildew. Chlorantraniliprole (DuPont Coragen; 18.4% AI; DuPont, Wilmington, DE), pyriproxyfen (Knack; 11.23% AI;

Valent U.S.A. LLC, Walnut Creek, CA) and pyrifluquinazon (PQZ; 20.2% AI; Nichino America Inc, Wilmington, DE) were sprayed on the squash plants for the control of whiteflies.

Flupyradifurone (Sivanto Prime; 17.09% AI; Bayer CropScience LP, St. Louis, MO) was sprayed on the squash for the control of squash bugs and whiteflies. Dinotefuran (Venom Insecticide; 70% AI; Valent U.S.A. LLC, Walnut Creek, CA) was sprayed on the plants for the control of squash bugs. Bifenthrin (Bifenture EC; 25.1% AI; United Phosphorus Inc, King of Prussia, PA) was sprayed on the plants for the control of squash bugs and squash vine borers.

Experimental design and treatment application

Spring trial. The experimental design utilized for the spring trials was a completely randomized design with four replicates per treatment. The treatments were different fumigant products, a RKN-resistant bell pepper cultivar cv. Carolina Wonder and an untreated check. The fumigant and untreated check treatments were planted with a susceptible pepper cultivar cv. Aristotle. Each experimental plot had a length and width of 51.82 m and 0.91 m, respectively. Treatments and application rates were as follows (Table 2.1): (i) 281 L/ha of allyl isothiocyanate (Dominus; 96.3% AI; Tri-Est Ag. Group, Tifton, GA), (ii) 196 L/ha of 59.6% chloropicrin + 39% 1,3-Dichloropropene (Pic60; 98.6% AI; Tri-Est Ag. Group, Tifton, GA), (iii) 140 L/ha of 1,3-Dichloropropene (Telone II; 97.5% AI; Tri-Est Ag. Group, Tifton, GA), (iv) Carolina Wonder pepper, and (v) untreated check. Fumigation was done on 19 March and 18 March in 2019 and 2020, respectively; at least three weeks prior to transplanting. The fumigants were injected into the soil at a depth of approximately 30.5 cm from the top of the bed through three backswept chisels spaced 25.4 cm apart and attached to a plastic mulch layer. White-on-black impermeable low-density polyethylene (LDPE) film mulch was placed over beds immediately after fumigation using a commercial tractor drawn bed-shaper. A single row of drip tape with

30.5 cm spacing between emitters was placed underneath the plastic mulch at a depth of 2.54–5.08 cm. Pepper seeds were sown in 128-seedling capacity styrofoam trays containing a mixture of sphagnum peat moss and perlite (Pro-Mix Bx Mycorrhizae; Premier Tech Horticulture, Quakertown, PA) in the greenhouse at 25-28 °C. After 6 weeks, the seedlings were transplanted by hand on 16 April and 27 April in 2019 and 2020, respectively to holes made on the plastic beds spaced 60.96 cm; each plot received a total of 85 plants.

Average soil temperature at a depth of 20 cm during the spring trial from the time of transplant to harvest were 25.6 °C and 25.54 °C in 2019 and 2020, respectively. The average precipitation for 2019 and 2020 were 218.2 mm and 250.5 mm, respectively. Average maximum and minimum air temperature in 2019 were 31.3 °C and 19.2 °C, and in 2020 were 29.65 °C and 18.78 °C, respectively (<http://www.georgiaweather.net/>).

Summer trial . This trial was conducted during the summer on the same field and plastic beds that were used for the spring trial. At the end of the spring trial, the leftover pepper plants were mowed and glyphosate (Credit 41 Extra; 41% AI; Nufarm Inc, Alsip, IL) was sprayed on the whole field at 2.3 L/ha. This was followed by paraquat dichloride (Paraquat concentrate; 43.2% AI; Source Dynamics LLC, Yuma, AZ) a week later at the rate of 2.1 L/ha ounces/acre and the fields were subsequently left fallow for one to two weeks. The summer trial utilized a split-plot design with four replications where the treatments (except the untreated check treatment) on pepper in the spring were the main plots and the non-fumigant nematicide treatments on squash the subplots. Plots were 7.92 m long and 0.91 m wide with 3.05 m alley between plot ends. Same treatment plots were utilized for the 2019 and 2020 trial. Squash (*Cucurbita pepo* Linnaeus) cv. Spineless Beauty seeds were planted in the greenhouse as

described above for two weeks and then 13 seedlings were transplanted to each plot on 29 July and 13 August in 2019 and 2020, respectively.

Non-fumigant nematicides were applied at the time of transplanting except fluensulfone which was applied seven days earlier and Majestene which had an additional application 21 days after the first application. The nematicides were applied via drip-irrigation using a CO₂ pressurized tank. Each nematicide was mixed in a 3-liter bottle and applied individually to each treatment plot. Prior to nematicide application, the beds were irrigated for two hours to allow for adequate bed moisture increasing the mobility of the nematicide in the soil. After the chemigation event, the irrigation was left running for 30 minutes to flush out any remaining nematicides from the drip tapes. Treatments and rates were (Table 2.1): (i) 18.7 + 9.35 L/ha of *Burkholderia* spp. strain A396 (Majestene; 94.46% AI; Marrone Bio Innovations, Davis, CA), (ii) 5.85 L/ha of fluensulfone (Nimitz; 40% AI; ADAMA Agricultural Solutions Ltd., Raleigh, NC), (iii) 2.24 L/ha of fluazaindolizine (Salibro; 41.15% AI; Corteva Agriscience, Indianapolis, IN), (iv) 0.48 L/ha of fluopyram (Velum Prime; 41.5% AI; Bayer CropScience, Research Triangle Park, NC), and (v) 4.68 L/ha of oxamyl (Vydate L; 24% AI; Corteva Agriscience, Indianapolis, IN).

Average soil temperature at a depth of 20 cm during the summer trial from the time of transplant to harvest were 28.49 °C and 26.62 °C in 2019 and 2020, respectively. The average precipitation for 2019 and 2020 were 173.5 mm and 235.2 mm, respectively. Average maximum and minimum air temperature in 2019 were 34.7 °C and 21.79 °C, and in 2020 were 29.96 °C and 20.07 °C, respectively.

Data collection

Spring trial. Soil PPN population density, root galling, stand count, plant vigor and yield were evaluated in both years. Soil samples were collected for nematode analysis prior to fumigation, at mid-season [39 days after transplanting (DAT)], and at the termination of the trials on 5 July and 13 July in 2019 and 2020, respectively. At each sampling time of the plots, soil cores (1.9 cm diameter and 20 cm depth) were collected close to the rooting zones of randomly selected plants. Ten soil cores were collected prior to fumigation and at harvest while five cores were collected at midseason. The soil cores were mixed thoroughly and nematodes were extracted from a 100-cm³ subsample by the sieving-centrifugation method (Jenkins, 1964). PPN were identified to the genus level and counted under the inverted microscope.

Five and ten root systems were randomly collected from each plot at mid-season and harvest, respectively, and evaluated for gall severity using an index of 0 to 5 where 0 = no galls seen on roots, and 5 = >75% galls on the root (Hussey and Janssen, 2002). Stand counts were done 28 and 56 DAT by counting the number of healthy plants in each plot. The vigor rating of the plants in each plot were measured with a Trimble GreenSeeker handheld crop sensor at 56 DAT. The sensor device measures reflected infrared rays from the green canopy of the plants and provide a normalized difference vegetation index (NDVI) value using a scale that ranges from 0.00 to 0.99. A higher NDVI value indicates more vegetative growth, and this translates to mean greater plant vigor (Desaeger and Watson, 2019). Pepper fruits were harvested by hand from ten randomly selected plants whose roots were later used for evaluating root gall severity. The fruits were subsequently graded into large-sized (greater than 9.4 cm in diameter), medium-sized (8.9–9.4 cm in diameter) and small-sized (less than 8.9 cm in diameter) fruits using a grader (Tew Manufacturing Corp. Penfield, N. Y. 14526) and then counted and weighted.

Summer trial. Trials were terminated on 16 September and 16 October in 2019 and 2020, respectively. Soil population density of PPN was determined as described previously at harvest from 5 soil cores. Five squash roots were randomly collected at the end of the season for assessing root gall severity as described previously. Stand count and vigor ratings using the Trimble GreenSeeker handheld crop sensor were done 21 and 42 DAT. Two (35 and 49 DAT) and three (41, 53 and 62 DAT) harvest dates were performed in 2019 and 2020, respectively and fruits with the size greater than 15.24 cm were harvested and weighed.

Statistical analysis

Data were analyzed with ANOVA using PROC GLIMMIX within SAS® 9.4 and means were separated by Tukey's adjustment for multiple comparisons test at $P < 0.05$. Data from the spring trial were analyzed as a completely randomized design and the treatments were treated as a fixed effect in a one-way ANOVA analysis. The data from the summer trial were analyzed as a split-plot design and the spring and summer treatments were treated as fixed effects in a two-way ANOVA analysis. Data were combined across years where no year \times treatment effect existed ($P < 0.05$). Before analysis, the residual plot of each variable datum was examined to ensure they satisfied the normality and homoscedasticity assumptions. Outliers in the data were removed using the studentized residual and Lund's test range of -3.4 to +3.4. Nematode count data were transformed using $\log_{10}(x + 1)$ to satisfy the normality assumptions.

Results

Spring trial. There was no treatment and year \times treatment effect on stand count and plant vigor (Table 2.2). Year \times treatment effect had no impact on the large-sized fruit yield and medium-sized fruit yield (Table 2.2) but significantly impacted the small-sized fruit yield (Table

2.3). Large-sized fruit yield was affected by the treatments with Pic60 having a higher ($P < 0.05$) yield than the 1,3-D and resistant cultivar treatments; however, there was no difference between Pic60 and the AITC and untreated check treatments. The treatments did not affect the medium-sized fruit yield (Table 2.2). Small-sized fruit yield was affected by the treatments during the trial conducted in 2020 but not in 2019. The resistant cultivar treatment had a higher ($P < 0.05$) yield than the AITC and Pic60 treatments in 2020; however, there was no difference between the resistant cultivar, 1,3-D and untreated check treatments.

Soil population density of *M. incognita* J2 was not impacted by the year \times treatment effect at different timepoints. The nematode numbers in the soil were similar among the treatments before fumigation ($P = 0.9944$) and only differed at mid-season ($P = 0.0231$) and harvest ($P = 0.0005$) (Table 2.2). Before fumigation, the average nematode numbers ranged from 6 to 13 J2/100 cm³ of soil. At midseason, only 1,3-D treatment significantly reduced the *M. incognita* populations as compared to the untreated check. However, at harvest, only the resistant cultivar significantly reduced the J2 numbers compared to the untreated check.

There was no interaction effect between year and treatment on root galling at harvest (Table 2.2); however, an interaction existed at mid-season (Table 2.3). The treatments affected root galling at harvest in both years (Table 2.2) and only affected mid-season root galling in 2019 (Table 2.3). At mid-season of 2019 and harvest of both years, all treatments except AITC significantly reduced root galling compared to the untreated check. Soil population densities of *Paratrichodorus* spp., *Helicotylenchus* spp. and *Mesocriconema* spp. were not impacted by the treatments and year \times treatment effect (data not shown).

Summer trial. There was a year \times non-fumigant effect on squash vigor at 21 ($P = 0.0110$) and 42 DAT ($P = 0.0070$), and on the yield ($P = 0.0418$) (Table 2.4). Non-fumigant

treatments influenced squash vigor at 21 DAT in 2019 with plots treated with fluensulfone having better ($P < 0.05$) vigor rating than fluopyram and fluazaindolizine. Squash plants vigor in Majestene-treated plots did not differ from other treatments, while plots treated with oxamyl had vigor rating only better than the fluazaindolizine treatment. At 42 DAT in 2019, plots treated with Majestene and oxamyl did better ($P < 0.05$) than fluensulfone and fluazaindolizine but had vigor rating equivalent to fluopyram. Non-fumigant treatments had no impact on plant vigor at any time point in 2020. Fumigant treatments only had a significant effect on plant vigor at 42 DAT in 2020. Plants treated with Pic60 had higher ($P < 0.05$) vigor than those in the 1,3-D treatments. The vigor rating of plants in the resistant cultivar and AITC treatments did not differ from that of Pic60 and 1,3-D treatments.

There was no fumigant \times non-fumigant effect for squash fruit yield in 2019 and 2020 (Table 2.4). The fumigant treatments influenced the fruit yield in 2020 with Pic60 having a better ($P < 0.05$) yield than all the other treatments. Non-fumigant treatments had a significant impact on fruit yield in 2019. Majestene and fluensulfone treatments had significantly higher fruit yield than fluazaindolizine. The stand count of the squash plants was not impacted by the non-fumigant treatments at 21 DAT; however, it was higher ($P < 0.05$) in plots treated with fluensulfone and Majestene than fluazaindolizine at 42 DAT (Table 2.5).

The fumigant treatment did not impact root galling of the squash plant; however, non-fumigant treatments significantly affected root galling with fluensulfone having lower ($P < 0.05$) gall severity when compared to oxamyl treatment (Table 2.5). *M. incognita*, *Paratrichodorus* spp. and *Mesocriconema* spp. soil population densities at harvest were neither influenced by the fumigant nor non-fumigant treatments (Table 2.5). Soil population densities of *Helicotylenchus* spp. at the end of the season was affected by the fumigant treatments in 2019 ($P = 0.0029$). Pic60

treatment significantly reduced the soil population of *Helicotylenchus* spp. relative to AITC and the resistant cultivar treatments (Table 2.4).

Discussion

A good decision on the fumigant and non-fumigant combination to use in a double or multi-cropping plasticulture system will help growers have proper control of the RKN and boost crop yield. In the present study, for the spring trials, Pic60, 1,3-D and the resistant cultivar reduced root galling in pepper at the end of the season. Similar results have been reported in previous research where each of the treatment has been observed to be effective in reducing the number of root galls. Morris et al. (2015) in a two-year field trial involving 1,3-D applied at a rate of 112 liters/ha in tomato reported a significant reduction in root galling compared to the untreated check. Thies et al. (2008) noted the resistant cultivar Carolina Wonder reduced root galling by 78% compared to its susceptible parent, Yolo Wonder B. Desaegeer and Watson (2019) reported that tomato plots with a main factor treatment of 87.4 kg/ha of 1,3-D and 134 kg/ha of chloropicrin (Pic60) resulted in significantly lower root galling at 65 and 102 days after planting than the untreated check plots.

The soil population of *M. incognita* at the end of the season was only greatly reduced in the resistant cultivar plot. A previous study (Thies et al., 2004) evaluating RKN-resistant (Charleston Belle) and susceptible (Keystone Resistant Giant) peppers in a double-cropping system reported a low soil population of *M. incognita* in the resistant cultivar than the susceptible treatment at the end of the trial. However, unlike this study, the susceptible cultivar had no chemical treatment along with it.

In our study, the resistant pepper (Carolina Wonder) also had the poorest fruit yield despite significantly reducing the root gall index and *M. incognita* numbers in the soil. In a previous study, no difference in pepper yield was observed between Carolina Wonder and its susceptible parent (Yolo Wonder B) in the presence or absence of a fumigation effect of methyl bromide (Theis et al., 2008). However, no data is available comparing the yield of Carolina Wonder alongside the susceptible cultivar (Aristotle) used in this study and the chemical treatment.

Pic60 showed a greater trend towards increasing crop yield than 1,3-D and the resistant cultivar. This advantage might be due to the fact that the mixture of 1,3-D (a nematicide) and chloropicrin (a fungicide) was not only effective against PPN but also suppressed other soilborne pathogens such as *Verticillium dahliae* and *Fusarium oxysporum* (Klose et al., 2007; Slusarski and Spotti, 2016) as well as some weeds (Boyd et al., 2017; Nnamdi et al., 2020; Gilreath and Santos, 2004). In our trials, we also have noticed the presence of southern blight disease caused by *Athelia rolfsii* that could have been treated better with Pic60 than 1,3-D, resulting in higher yields.

Another nematicide application for subsequent crops grown on the plastic is essential for PPN control. The importance of this application is further necessitated if the first crop is planted in the spring and the second crop in the summer because RKN populations increase rapidly in the summer than spring due to higher soil temperature which allows faster completion of the nematode life cycle (Desaeger and Csinos, 2006; Ploeg and Maris, 1999). Hajihassani et al. (2019a) stated that *Meloidogyne* spp. complete three to four life cycles on fruiting vegetables such as pepper in South Georgia with high temperatures and abundant rainfall, resulting in higher nematode populations in the soil. Therefore, drip application of non-fumigant nematicides

is recommended for RKN control in subsequent crops (Desaeger and Csinos, 2006). Drip-applied fumigants are not the best option for this purpose because of the uneven distribution of chemicals in the bed. This has been attributed to rapid volatilization of the fumigants caused by planting holes from the first crop and limited movement of the fumigants from the drip tape which is a single point injection at the surface of the soil (Desaeger and Csinos, 2006; Desaeger et al., 2008). Also, the buffer zone requirement for fumigants and long plant-back date makes non-fumigant nematicides a better alternative.

In the summer trials, the untreated check plots from the spring trial were ignored and an untreated check plot was not part of the summer treatments. Fluensulfone reduced root galling better than oxamyl; however, this did not translate into fluensulfone treated plots having a better yield than oxamyl plots. Previous studies have noted both nematicides to have the same impact on crop yield and same level of root galling control in tomatoes (Desaeger and Watson, 2019) and watermelon (Hajihassani et al. 2020). On-farm trials conducted by Khanal and Desaeger (2020) reported fluensulfone to reduce root galling in cucumber greater than oxamyl, but both nematicides had the same level of root galling control in cantaloupe. They also noted that both fluensulfone and oxamyl had the same impact on yields of cucumber and cantaloupe.

In our trials, fluazaindolizine, which reduced root galling equivalent to fluensulfone, had lower yield in the 2019 trial. This might be due to poor plant vigor early in the growing season and eventual loss of plant stands during the trial. Majestene and fluopyram had the same level of RKN control and squash yield as that of fluensulfone and oxamyl. A previous study (Desaeger and Watson, 2019) compared the effectiveness of fluensulfone, fluopyram, fluazaindolizine and Majestene in the plasticulture production of tomato and obtained no difference in root galling and crop yield between the nematicides. However, in that study, these nematicides were subplot

treatments with fumigation being used as the main plot treatment in the same cropping season (Desaeger and Watson, 2019). When looking at the fumigant effect only on the summer trial in the present study, Pic60 had a better yield than AITC, 1,3-D and the resistant cultivar.

Overall, Pic60, 1,3-D and the resistant cultivar (cv. Carolina Wonder) were ideal options for RKN control in pepper when planted in the spring. For fruit yield, Pic60 showed the greatest trend towards yield increase than other treatments, while for the summer trial, any of the non-fumigant nematicides led to similar efficacy. A combination of Pic60 and any of the non-fumigant nematicides examined should be a good fit for RKN management in a pepper-squash plasticulture cropping system.

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Table 2.1

Overview of the treatments, application rates and timing of nematicides.

Treatment	Season	Product application rate (L/ha)	Application timing
Allyl isothiocyanate (AITC)	Spring	281	21 days before transplanting (DBT)
59.6% chloropicrin + 39% 1,3-D (Pic60)	Spring	196	21 DBT
1,3-Dichloropropene (1,3-D)	Spring	140	21 DBT
Resistant cultivar (Carolina Wonder)	Spring	-	-
Untreated check	Spring	-	-
<i>Burkholderia</i> spp. strain A396 (Majestene)	Summer	18.7 + 9.35	At transplant + 21 days after transplanting
Fluensulfone	Summer	5.85	7 DBT
Fluazaindolizine	Summer	2.24	At transplant
Fluopyram	Summer	0.48	At transplant
Oxamyl	Summer	4.68	At transplant

Trial dates from transplant to termination:

Spring 2019: 16 April – 5 July; Summer 2019: 29 July – 16 September.

Spring 2020: 27 April – 13 July; Summer 2020: 13 August – 16 October.

Table 2.2

Effect of soil fumigation and resistant pepper (spring treatments) on plant vigor, stand count, fruit yield, root-knot galling at harvest and population densities of *Meloidogyne incognita* second-stage juveniles in 2019 and 2020.

Treatment	Vigor (56 DAT) ^a	Stand count ^b		Fruit yield ^c		RGI at harvest ^d	<i>M. incognita</i> /100 cm ³ of soil		
		28 DAT	56 DAT	Large- sized	Medium -sized		Before fumigation	At midseason ^e	At harvest ^f
AITC	0.29 a ^g	78.88 a	72.25 a	1.75 ab	2.15 a	1.1 ab	6.75 a	1.25 ab	135.5 a
Pic60	0.37 a	81.88 a	78.63 a	3.15 a	2.89 a	0.28 c	8 a	1.63 ab	37.25 ab
Resistant cultivar	0.30 a	79.13 a	73.50 a	0.2 c	1.29 a	0.1 c	6 a	1.88 ab	1.25 b
1,3-D	0.29 a	78.13 a	70.50 a	1.31 bc	2.48 a	0.41 bc	13 a	0.25 b	38 ab
Untreated check	0.29 a	77.63 a	72.63 a	1.87 ab	2.21 a	2.24 a	10.5 a	6.75 a	226.25 a
<i>P</i> -value									
Year (Y)	<0.0001	<0.0001	<0.0001	0.6929	0.7820	0.3649	0.0005	<0.0001	0.6095
Treatment (T)	0.1719	0.5791	0.2264	0.0001	0.1708	<0.0001	0.9944	0.0231	0.0005
Y × T	0.5294	0.1926	0.0837	0.0528	0.1708	0.4890	0.8199	0.0830	0.8834

Allyl isothiocyanate (AITC); 59.6% chloropicrin + 39% 1,3-D (Pic60); Resistant cultivar (cv. Carolina Wonder); 1,3-Dichloropropene (1,3-D); days after transplanting (DAT); Root gall index (RGI).

^a Vigor rating of the pepper plants were measured with the aid of a handheld crop sensor using a scale of 0 – 1.

^b Stand count was done by counting the number of healthy plants in each plot.

^c Fruit yield is in kg/10 plants; fruits greater than 9.4 cm in diameter were graded as large-sized and fruits with 8.9 – 9.4 cm in diameter were graded as medium-sized (Tew Manufacturing Corp. Penfield, N. Y. 14526).

^d Root gall rating was done using an index of 0 to 5 with 0 being no galls seen on roots and 5 being >75% of root system galled (Hussey and Janssen, 2002).

^e Midseason = 39 days after transplanting.

^f End of the season = 77 days after transplanting.

^g Data are the means of two years and four replications (n = 8). Means with same letter within a column are not significantly different ($P < 0.05$).

Table 2.3

Effect of soil fumigation and resistant pepper (spring treatments) on root galling caused by *Meloidogyne incognita* at mid-season and small-sized fruit of pepper in 2019 and 2020.

Treatment	RGI at mid-season ^a		Small-sized fruit yield ^b	
	2019	2020	2019	2020
AITC	0.55 ab ^c	0.15 a	0.42 a	4.40 b
Pic60	0.05 b	0.00 a	0.81 a	3.98 b
Resistant cultivar	0.15 b	0.00 a	0.59 a	6.25 a
1,3-D	0.05 b	0.10 a	0.47 a	4.76 ab
Untreated check	1.05 a	0.25 a	0.30 a	5.36 ab
<i>P</i> -value	<0.0001	0.3000	0.1029	0.0083

Allyl isothiocyanate (AITC); 59.6% chloropicrin + 39% 1,3-D (Pic60); resistant cultivar (cv. Carolina Wonder); 1,3-Dichloropropene (1,3-D); Root gall index (RGI).

^a Root gall rating was done using an index of 0 to 5 with 0 being no galls seen on roots and 5 being >75% of root system galled (Hussey and Janssen, 2002). Midseason = 39 days after transplanting.

^b Fruit yield is in kg/10 plants; fruits less than 8.9 cm in diameter were graded as small-sized (Tew Manufacturing Corp. Penfield, N. Y. 14526).

^c Data are the means of one year and four replications ($n = 4$). Means with same letter within a column are not significantly different ($P < 0.05$).

Year data are not combined because there was a year \times treatment effect (RGI at mid-season: $P = 0.0068$; small-sized fruit yield: $P = 0.0019$).

Table 2.4

Effect of soil fumigation and resistant pepper (spring treatments) and non-fumigant nematicides (summer treatments) on squash vigor, fruit yield and *Helicotylenchus* spp. in 2019 and 2020.

Treatment	Vigor ^a				Yield (kg/plot) ^b		<i>Helicotylenchus</i> count/100 cm ³ of soil	
	2019		2020		2019	2020	2019	2020
	21 DAT	42 DAT	21 DAT	42 DAT				
<i>Fumigant</i> ^c								
Pic60	0.44 a	0.59 a	0.30 a	0.61 a	27.58 a	29.92 a	0.11 c	0.05 a
AITC	0.43 a	0.51 a	0.15 a	0.45 ab	21.64 a	12.82 b	3.53 a	0.05 a
Resistant cultivar	0.44 a	0.55 a	0.14 a	0.46 ab	27.51 a	12.50 b	2.20 ab	0.25 a
1,3-D	0.41 a	0.57 a	0.12 a	0.40 b	22.28 a	11.46 b	0.32 bc	0.00 a
<i>Non-fumigant</i> ^d								
Majestene	0.42 abc	0.62 a	0.19 a	0.48 a	28.51 a	17.52 a	0.93 a	0.19 a
Fluensulfone	0.53 a	0.50 b	0.19 a	0.50 a	27.50 a	17.34 a	0.50 a	0.13 a
Fluazaindolizine	0.33 c	0.50 b	0.16 a	0.47 a	18.55 b	15.57 a	1.50 a	0.06 a
Fluopyram	0.40 bc	0.56 ab	0.16 a	0.45 a	24.55 ab	16.29 a	3.33 a	0.00 a
Oxamyl	0.48 ab	0.63 a	0.19 a	0.48 a	25.60 ab	16.66 a	1.23 a	0.06 a
<i>P-value</i>								
Fum	0.9865	0.3757	0.0719	0.0295	0.4279	0.0019	0.0029	0.2513
Non	0.0009	<0.0001	0.3199	0.7451	0.0272	0.8548	0.0892	0.6888
Fum × non	0.4391	0.1213	0.2053	0.5856	0.1639	0.6268	0.6426	0.7575

Fumigant (Fum); Non-fumigant (Non); Allyl isothiocyanate (AITC); 59.6% chloropicrin + 39% 1,3-D (Pic60); Resistant cultivar (cv. Carolina Wonder); 1,3-Dichloropropene (1,3-D); *Burkholderia* spp. strain A396 (MAJ); days after transplanting (DAT).

^a Vigor rating of the pepper plants were measured with the aid of a handheld crop sensor using a scale of 0 to 1.

^b Yield was calculated as the sum of two and three harvest dates for 2019 and 2020, respectively.

^c Data are the means of four replications ($n = 4$).

^d Data are the means of sixteen replications ($n = 16$).

Means with same letter within each treatment level column are not significantly different ($P < 0.05$).

Year data are not combined because there was a year × non-fumigant effect (vigor at 21 DAT: $P = 0.0110$; vigor at 42 DAT: $P = 0.0070$; yield: $P = 0.0418$, *Helicotylenchus* count: $P = 0.0001$).

Table 2.5

Effect of soil fumigation and resistant pepper (spring treatments) and non-fumigant nematicides (summer treatments) on root-knot galling of squash caused by *M. incognita*, stand count, and soil population densities of second-stage juveniles of *Meloidogyne incognita*, *Paratrichodorus* spp. and *Mesocriconema* spp. in 2019 and 2020.

Treatment	RGI at harvest ^a	<i>M. incognita</i> count/100 cm ³ of soil	Stand count ^b		<i>Paratrichodorus</i> count/100 cm ³ of soil	<i>Mesocriconema</i> count/100 cm ³ of soil
			21 DAT	42 DAT		
<i>Fumigant^c</i>						
Pic60	1.21 a	65.51 a	11.97 a	11.44 a	2.62 a	1.92 a
AITC	1.61 a	50.68 a	11.59 a	10.68 a	1.41 a	2.73 a
Resistant cultivar	0.76 a	10.40 a	12.08 a	11.33 a	2.18 a	11.13 a
1,3-D	1.28 a	40.77 a	11.64 a	10.64 a	4.87 a	5.23 a
<i>Non-fumigant^d</i>						
Majestene	1.32 ab	42.90 a	12.19 a	11.68 a	2.84 a	8.71 a
Fluensulfone	0.87 b	38.84 a	11.66 a	11.63 a	1.97 a	1.72 a
Fluazaindoline	1.16 ab	30.94 a	11.59 a	10.16 b	3.34 a	6.06 a
Fluopyram	1.06 ab	55.48 a	11.61 a	10.58 ab	4.65 a	5.52 a
Oxamyl	1.66 a	39.76 a	12.1 a	11.1 ab	1.0 a	4.66 a
<i>P-value</i>						
Year	0.3212	0.7548	<0.0001	<0.0001	<0.0001	0.2396
Fum	0.5695	0.3748	0.4741	0.2772	0.4544	0.1817
Fum × year	0.8849	0.8241	0.3953	0.7751	0.8320	0.8503
Non	0.0231	0.3311	0.4784	0.0034	0.4299	0.6790
Non × year	0.9599	0.7477	0.9791	0.1062	0.7004	0.7360
Fum × non	0.1416	0.5523	0.7677	0.7540	0.6751	0.6028
Fum × non × year	0.9046	0.5133	0.7032	0.6465	0.9398	0.6482

Fumigant (Fum); Non-fumigant (Non); Allyl isothiocyanate (AITC); 59.6% chloropicrin + 39% 1,3-D (Pic60); Resistant cultivar (cv. Carolina Wonder); 1,3-Dichloropropene (1,3-D); *Burkholderia* spp. strain A396 (MAJ); Root gall index (RGI).

^a Root gall rating was done using an index of 0 to 5 with 0 being no galls seen on roots and 5 being >75% of root system galled (Hussey and Janssen, 2002).

^b Stand count was done by counting the number of healthy plants in each plot.

^c Data are the means of two years and four replications each ($n = 8$).

^d Data are the means of two years and sixteen replications each ($n = 32$).

Means with same letter within each treatment level column are not significantly different ($P < 0.05$).

CHAPTER 3

EFFECT OF DEEP APPLICATION OF NON-FUMIGANT NEMATOCIDES ON *MELOIDOGYNE INCOGNITA* IN A TOMATO PLASTICULTURE SYSTEM

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Abstract

Root-knot nematodes (RKN; *Meloidogyne* spp.) is detrimental to vegetable crops in the Southeastern USA due to the serious damage it causes. To manage this nematode, the placement of a single or double drip tape at a depth of 2.54 cm below the plastic mulch to aid in chemigation is a common practice in vegetable plasticulture systems. However, nematicide application at this depth might offer little or no control of *M. incognita* populations existing deep in the soil profile that may move upward and infect host crops later in the season. A field trial was conducted in the spring of 2019 and 2020 and summer of 2021 to evaluate the best combination of drip tapes [Surface and Sub-surface Drip Tapes (SSDT) vs only Surface Drip Tape (SDT)] and nematicides (fluensulfone, fluazaindolizine, and fluopyram) for *M. incognita* control in tomato. Tomato (cv. Red Bounty) was used in 2019 and 2020 trials, while tomato (cv. Roadster) was used in 2021 trials. Surface and sub-surface drip tapes were placed at a depth of 5.08 and 30.48 cm below the soil surface, respectively. In 2019, the three nematicides reduced ($P < 0.05$) both root galling and *M. incognita* populations compared with 2020, where no effect was observed. Root galling in 2021 was only significantly reduced by fluensulfone and fluazaindolizine treatments, while the nematode population was only reduced by fluensulfone treatments. There was no drip tape effect and drip tape by nematicide effect on the root galling and the nematode population density at the end of the season in all the trials. Tomato yield of the trials was not impacted by the drip tape placement, nematicide treatments and drip tape by nematicide effect. This study shows that the drip tape utilized for nematicide application plays no role in the control of *M. incognita* in a single cropping system. However, this technique might be effective in multi-cropping plasticulture systems and requires further investigation.

Introduction

The use of plastic mulch in vegetable cultivation is relatively common in the Southeastern USA, and this aids plant growth by boosting temperature absorption and retention of soil moisture (Hajihassani, 2018; Lament, 1993; Morris et al., 2015). Multiple crops are grown between two to several seasons on a single application of plastic mulch to eliminate the cost of applying new plastic each time a crop is grown and removing the old plastic from the field (Morris et al., 2015). This multi-cropping system on the same plastic mulch encourages the buildup of plant-parasitic nematodes (PPN) (Hajihassani, 2018) in particular root-knot nematodes (RKN; *Meloidogyne* spp.) that are extensively associated with vegetable production systems in South Georgia and cause significant yield losses (Hajihassani et al., 2019; Marquez et al., 2019).

Chemigation is often used to manage PPN in plasticulture systems, and involves nematicide application via drip irrigation system (Desaeger and csinos, 2006). A single or double drip tapes are usually placed at a depth of 2.54 to 7.62 cm below the surface of the soil when laying the plastic mulch over raised beds. The drip tape is also used to deliver fertilizers and other pesticides (Lamont et al., 2016; Kemble et al., 2019). Nematicide application, however, at the upper soil surface may only affect PPN populations associated with the roots systems. This is because the effect of nematicides is restricted to a few weeks after planting (Colyer et al. 1997), and nematode populations present at lower depth may move upward and infect plants later in the season (Noling, 2016). The vertical distribution of PPN (including *M. incognita*) in the soil up to a depth of 45 cm has been reported McSorley and Dickson (1990).

The last decade has seen the discovery of new non-fumigant nematicide chemistries and the introduction of products with novel modes of action in the market. For example, fluensulfone

was registered for use in vegetables as Nimitz® by ADAMA in 2014 (Gine, 2016) to control PPN. In addition, fluopyram, a known fungicide, was discovered to kill PPN and registered as a nematicide (US EPA, 2016). More recently, fluazaindolizine was discovered and is currently undergoing final registration and regulatory procedure before introduction to the pesticide market (Lahm et al., 2017; Thoden and Wiles, 2019). Greenhouse, microplot, and field trials have demonstrated the efficacy of fluensulfone, fluopyram and fluazaindolizine against PPN (Becker et al., 2019; Hajihassani et al., 2019; Jones et al., 2017; Nnamdi et al., 2022). However, to the best of our knowledge, no study has yet evaluated the potential impact of these non-fumigant nematicides when applied deep in the soil profile to manage RKN in vegetables.

The objective of this research was to determine the effect of fluensulfone, fluazaindolizine, and fluopyram applied by different drip tape techniques [Surface and Sub-surface Drip Tapes (SSDT) vs. only Surface Drip Tape (SDT)] in the control of RKN and their impact on tomato yield.

Materials and methods

Site description and land preparation. This field experiment was carried out at the University of Georgia (UGA) Horticultural Hill Farm in the spring of 2019 and 2020 and at the UGA Black Shank Farm in the summer of 2021. The fields were areas of continuous vegetable production with Tifton sandy soil and natural infestation of *M. incognita*. Okra (*Abelmoschus esculentus* Linnaeus) and hairy vetch (*Vicia villosa* Roth) were consecutively grown in the fall and winter preceding each spring planting season in 2019 and 2020 to increase the soil population density of the RKN. The fields were harrowed and rototilled prior to the trial establishment each year, and 10-10-10 NPK rainbow granular fertilizer was incorporated into the

soil. A pre-emergence herbicide, Dual Magnum (Syngenta, Wilmington, DE), was applied on 2 April, 1 May, and 14 July in 2019, 2020, and 2021, respectively. Glyphosate was sprayed in between the rows and at the ends of the plot using a hooded sprayer every two weeks. Fungicide and insecticide applications were also made during the season as required by the plant. All pesticides and fertilizer applications were done following the Georgia pest management handbook and the Southeastern U.S. vegetable crop handbook (Kemble et al., 2021).

Experimental design and treatment applications. A split-plot randomized complete block design was utilized for this study, with the type of drip application (SSDT vs. SDT) being the main plot and the nematicides (fluensulfone, fluazaindolizine, and fluopyram) and control treatments the sub-plot. Nematicide treatments and their application rates were as follows: (i) 5.85 L/ha of fluensulfone (Nimitz; ADAMA Agricultural Solutions Ltd., Raleigh, NC), (ii) 2.24 L/ha of fluazaindolizine (Salibro; Corteva Agriscience, Indianapolis, IN), (iii) 0.48 L/ha of fluopyram (Velum Prime; Bayer CropScience, Research Triangle Park, NC). The main plots and sub-plots had four replicates, and each sub-plot had a length of 8.23 m and width of 0.91 m in 2019 and 2020 fields trials. In 2021 field trials, the main plot and sub-plots had five replicates and each sub-plot had a length of 6.1 m and width of 0.91 m. The 2021 trial was repeated twice at the University of Georgia Black Shank farm at different locations. There was a 3.05 m alley between plot ends for all the trials.

A sub-soil surface rig enabled the insertion of the sub-surface drip tape at a depth of 30.48 cm in the soil. The surface drip tape was placed 5.1 cm below the soil's surface by a drip tape layer, and the plastic mulch was laid immediately afterward with the aid of the plastic mulch layer. The drip tape(s) for each plot was cut at its end and closed using locking fittings to prevent cross-contamination among treatments. The drip tapes had a spacing of 30.5 cm, and the mulch

utilized for this experiment was a white-on-black low-density polyethylene (LDPE) mulch. The plastic mulch was laid on 2 May, 5 May and 15 July in 2019, 2020 and 2021, respectively.

Tomato (*Solanum lycopersicum* Linnaeus) cv. Red Bounty was used in the 2019 and 2020 field trials while tomato cv. Roadster was used in the 2021 field trials. Seeds were planted in the greenhouse in 128-seedling capacity Styrofoam trays containing a mixture of sphagnum peat moss and perlite (Pro-Mix Bx Mycorrhizae; Premier Tech Horticulture, Quakertown, PA). After five weeks, the seedlings were transplanted to the field on 13 May, 1 June, and 28 July in 2019, 2020 and 2021, respectively. Plant to plant spacing in each plot was 61.0 cm; each sub-plot received a total of 13 plants in 2019 and 2020 field trials and 10 plants in 2021 field trials. Fluazaindolizine and fluopyram were applied at transplanting, while fluensulfone was applied seven days before transplanting. In 2019 and 2020 field trials, a CO₂ pressurized tank was used in pumping different 3-liter bottles containing each nematicide to a drip irrigation manifold. The manifold was set up to deliver each nematicide to the required treatment plot. A drip irrigation manifold was not used in the 2021; instead, the CO₂ tank was used in pumping each treatment 3-liter bottle mixture of the nematicide to the specific treatment plot. Prior to nematicide application in all the trials, the beds were irrigated for two hours to allow for adequate bed moisture, which would increase the mobility of the nematicide in the soil. After the chemigation event, the irrigation was left running for 30 minutes to flush out any remaining nematicides from the drip tapes. A single drip hole put out an average of 28.75 ml/min. In 2019 and 2020 field trials, the amount of water put out for each plot of SDT treatment before and after nematicide application was 93 and 23 liters, respectively. In 2021 field trials, 69 and 17 liters of water were put out before and after nematicide application for each plot of the SDT treatment. For SSDT treatments, the amount of water put out at each time point was double that of SDT treatment. A

liquid fertilizer (7-0-7) was applied to the plots every week using the drip irrigation system and this began two weeks post-transplant until harvest.

Average soil temperature at a depth of 20 cm during the trials from the time of transplant to harvest were 27.7 °C, 28.3 °C, and 27.49 °C in 2019, 2020, and 2021, respectively. The average precipitation for 2019, 2020 and 2021 was 192.3 mm, 203.5 mm, and 296.91 mm, respectively. The average maximum and minimum air temperature were 32.8 °C and 21.4 °C in 2019, 32.7 °C and 21.7 °C in 2020, and 30.82 °C and 20.72 °C in 2021 (<http://www.georgiaweather.net/>).

Data collection and statistical analysis. Soil samples were collected from each subplot for PPN analysis before nematicide application, at midseason [39 days after transplanting (DAT)], and at the termination of the trials on 26 July, 11 August, and 13 October in 2019, 2020, and 2021, respectively. Midseason soil sampling was not done in the 2021 field trials. At each soil sampling time of the subplots, five soil cores of 1.9 cm diameter and 20 cm depth were collected close to the rooting zones of randomly selected plants whose roots were initially used for root gall evaluation. Soil cores were mixed thoroughly, and nematodes were extracted from a 100-cm³ subsample by the sieving-centrifugation method (Jenkins, 1964). PPN were identified to the genus level and counted under an inverted microscope. Crop vigor of the plants in each subplot was evaluated at 28 and 56 DAT with a Trimble GreenSeeker handheld crop sensor. This device measures the normalized difference vegetation index (NDVI) of the plants and this value is calculated from the reflected infrared rays of the green canopy of the plants. Stand count of each plot was done at 28 and 56 DAT. Three and five tomato plants were randomly collected from each subplot at midseason (39 DAT) and termination of the trial, respectively, for root gall evaluation using a gall index of 0 to 5 where 0 = no galls seen on roots, and 5 = >75% galls on

the root (Hussey and Janssen, 2002). Midseason root gall evaluation was not done in the 2021 field trials. Mature tomato fruits were harvested by hand at the end of the season from each plot, and their weight and number were determined. 2021 field trial had a widespread infestation of tomato yellow leaf curl virus, and this resulted in poor yield of all the plants.

Experimental data were subjected to analysis of variance using PROC GLIMMIX (SAS® 9.4), and means were separated by Tukey's adjustment for multiple comparisons test ($P < 0.05$). The data were analyzed as a split-plot design, and the type of drip tape (SSDT vs. SDT) and nematicide treatments were treated as fixed effects in a two-way ANOVA analysis. 2019 and 2020 data were combined when no year \times treatment interaction existed ($P < 0.05$). The repeated trials for the 2021 field study were pulled together when no experiment \times treatment interaction existed. Before analysis, the residual plot of each variable datum was examined to ensure they satisfied the normality and homoscedasticity assumptions. Outliers in the data were removed using the studentized residual and Lund's test range of -3.4 to +3.4. $\text{Log}_{10}(x + 1)$ was used to transform the nematode count data to satisfy the normality assumptions.

Results

Plant vigor, stand count, and fruit yield of tomato cv. Red Bounty. None of the treatment levels or their interactions impacted crop vigor rated at 28 DAT, stand count at 28 and 56 DAT, fruit weight, and the total number of fruits (Table 3.1). Nematicide treatments affected the crop vigor at 56 DAT, with control plots having a better ($P < 0.05$) vigor rating than the fluensulfone treatment. The vigor rating of the control plots was however not different from that of fluazaindolizine and fluopyram plots.

Soil nematode population density and root galling of tomato cv. Red Bounty. Year data were not combined for root galling because there was a year \times nematicide interaction for both midseason ($P < 0.0001$) and end of season gall indices ($P < 0.0001$) (Table 3.2). In 2019, gall ratings were significantly lower in the nematicide treated plots than in the control plots at midseason and end of the season. Plants in the fluensulfone plots had less ($P < 0.05$) galling than the fluopyram plots at the end of the season of 2019; however, this was not significantly different from root galling of plants in the fluazaindolizine plots. Root gall index at midseason in 2020 had lots of zeros and could not be analyzed even after log transformation of the data, while any treatment levels or interaction effects did not influence the root gall index at the end of the season.

The average number of second-stage juvenile (J2) of *M. incognita* in the soil prior to 2019 and 2020 field trials was 6.43 and 0.13 J2/100 cm³ of soil, respectively. None of the treatment levels or their interactions influenced the soil population density of *M. incognita* before nematicide applications (Table 3.1). Year data were not combined for the soil population densities of *M. incognita* at midseason and end of the season because there was a year \times nematicide \times drip tape interaction at midseason ($P = 0.0489$) and year \times nematicide interaction at the end of season ($P < 0.0001$) (Table 3.2). In 2019, the nematicide treatments impacted the soil population density of *M. incognita* at the mid and end of the season. Fluazaindolizine did better ($P < 0.05$) than the control treatment at both timepoints in reducing *M. incognita* populations in the soil. Fluensulfone and fluopyram only did better ($P < 0.05$) than the control treatment in reducing the nematode population density at the end of the season.

Helicotylenchus spp. (spiral nematodes) counts at all time points (data not shown) and *Mesocriconema* spp. (ring nematodes) counts before nematicide application (Table 3.3) had lots

of zeros and could not be analyzed even after log transformation of the data. *Paratrichodorus* spp. (stubby-root nematodes) numbers in the soil at midseason were only influenced by the drip tape with SSDT having a higher ($P < 0.05$) nematode number than the SDT. *Mesocriconema* spp. counts at mid and end of the season were not impacted by any treatment levels or interactions.

Plant vigor, stand count, and fruit yield of tomato cv. Roadster. None of the treatment levels or their interactions influenced crop vigor rating at 28 and 56 DAT, stand count at 28 DAT, fruit weight, and the total number of fruits (Table 3.4). Nematicide treatments had an effect on stand count at 56 DAT, with control plots having a better ($P < 0.05$) stand count than the fluensulfone treatment. However, the stand count of the control plots was not different from that of fluazaindolizine and fluopyram plots.

Soil nematode population density and root galling of tomato cv. Roadster. Root-galling of tomato cv. Roadster was only impacted by the nematicide treatments. Control and fluopyram treatments caused a higher ($P < 0.05$) root galling than fluensulfone and fluazaindolizine treatments. The average number of *M. incognita* J2 in the soil before initiation of the field trials in 2021 was 10.28 J2/100 cm³ of soil. Prior to nematicide application, SDT treatments had a significantly higher soil population density of *M. incognita* than SSDT treatments. However, at the end of the season, no difference was observed between the SDT and SSDT treatments. Only fluensulfone reduced ($P < 0.05$) the *M. incognita* population density at the end of the season. None of the treatment levels or interactions influenced the soil population density of *Paratrichodorus* spp. and *Mesocriconema* spp. prior to nematicide application and at the end of the season (Table 3.5).

Discussion

The main objective of this research was to evaluate the impact of nematicide application by SSDT vs. SDT in the control of *M. incognita*. Our results showed that the type of drip tape utilized plays no role in a single crop plasticulture system. SSDT treatment had no effect even in the 2021 field trial where a very RKN-susceptible variety (Roadster) was used, and the soil RKN population density before the trial initiation was high. Previous studies involving deep soil application of nematicide through drip tape had to do with fumigants and non-vegetable crops (Cabrera et al., 2012; Schneider et al., 2006; Schneider et al., 2009). Also, in some of these past research works, deep soil application of fumigants by drip tape was compared with shank application rather than surface drip tape application. In evaluating alternatives to methyl bromide for controlling PPN in the vineyard, Schneider et al. (2006) demonstrated that propargyl bromide, iodomethane + chloropicrin, and 1,3-dichloropropene + chloropicrin applied via drip tapes at a depth of 25 cm tended to be an adequate substitute to methyl bromide applied by shank injection at a depth of 56 cm. They also observed that RKN susceptible grapevines (Thompson Seedless) treated with sodium azide at a depth of 5 cm by drip tape did poorly in controlling RKN compared with grapevines treated with iodomethane + chloropicrin, 1,3-dichloropropene + chloropicrin, sodium azide at a depth of 25 cm by the drip tape and methyl bromide at a depth of 56 cm by the shank injection. Cabrera et al. (2012) reported that 1,3-dichloropropene + chloropicrin, and iodomethane + chloropicrin applied by the subsurface drip tape at a 20 to 25 cm depth had nematicidal activity comparable to methyl bromide applied by shank at a depth of 56 cm in grapevines. Schneider et al. (2009) compared fumigants applied by a shank at a depth of 25.4 and 45.7 cm and those applied by drip tape at a depth of 20.3 cm in a nut tree (*Prunus* spp.) nursery and observed shank-injected fumigants to have better control of nematodes than the

drip applied fumigants. However, the fumigants had the same level of weed control and yielded the same marketable trees irrespective of their application method.

Fluensulfone and fluazaindolizine reduced root galling in Roadster tomato in 2021, while all three nematicides reduced root galling in Red Bounty tomato in 2019. Previous studies have also reported the efficacy of these nematicides in the reduction of root galls. In microplot studies, Hajihassani et al. (2019) observed fluopyram, fluensulfone and fluazaindolizine to significantly reduce root galling in cucumber at inoculation densities of 1000, 5000, 10000, and 20000 *M. incognita* J2/microplot. They also noted that all three nematicides caused an increase in yield at inoculation densities of 10,000 and 20,000 nematodes/microplot and fluopyram caused yield increase at all inoculation densities. Becker et al. (2019) showed fluopyram, fluensulfone and fluazaindolizine to be efficacious in reducing root galling caused by *M. incognita* in carrot in multi-year field trials. They also documented a yield increase in fluensulfone and fluazaindolizine treatments in only one of the five field trials conducted.

Prior to the 2020 field trial, the soil population density of *M. incognita* was very low (0.125 J2/100 cm³ of soil), and this did not change throughout the season even for the control treatment where the average *M. incognita* at the end of the season was 2 J2/100 cm³ of soil. The low population density of *M. incognita* resulted in no root galling difference among treatments. Also, unlike the 2019 trial where the average *M. incognita* in the soil increased from 6 J2/100 cm³ at the beginning of the trial to 46 J2/100 cm³ at the end of the season, the average *M. incognita* in the soil at the end of the season in 2020 was only 2 J2/100 cm³ of soil. Increased soil RKN population at the end of the season has also been reported for some other nematicide field trials where there was a low soil population of RKN prior to the start of trials (Desaeger et al., 2017; Morris et al., 2015).

Tomato (cv. Red Bounty) is resistant to *M. incognita* (Regmi and Desaegeer, 2019); however, we observed a severe root galling (averaged 3.73 on a 0-5 scale) in the control treatment in 2019. Also, the average soil RKN population density at the end of the season was 134.75 J2/100 cm³ for Red Bounty tomato. Resistance breakdown of varieties (such as Charleston Belle and Carolina Wonder) have been documented at high soil temperatures of 28 and 32 °C (Thies and Fery, 1998). During the experiment in 2019 and 2020, the soil temperature was almost similar; however, a breakdown in resistance was only observed in 2019 and not in 2020. This may eliminate the possibility of soil temperature being the cause for the resistance breakdown of Red Bounty tomato in the 2019 field trial. Resistance breakdown has also been attributed to virulent or resistance-breaking populations of nematodes (Hajihassani et al., 2021; Kaloshian et al., 1996; Ornat et al., 2001; Tzortzakakis et al., 2005). There is a possibility that this might have caused the breakdown of the Red Bounty tomato resistance in 2019

The problem of incessant infestation by PPNs, particularly *Meloidogyne* spp. is a challenge for vegetables growers (Marquez et al., 2021). This propels the search for an effective management strategy that will reduce nematode damage, maximize crop yields while being financially worthwhile. Overall, this study shows SSDT application of nematicide has no impact on RKN management in a single cropping season. This technique, however, might be effective in multi-cropping plasticulture systems, where RKN population densities could be much higher around root zones, and populations of RKN at lower depths might have migrated to the root zone. Another conclusion drawn from this study is that the control of RKN in single cropping systems might need to be focused on nematode populations that exist in the top portion of the soil (rooting zone) and not those that live deep in the soil profile.

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Table 3.1

Effect of treatments on tomato vigor, stand count, fruit yield and the soil population density of second-stage juveniles of *Meloidogyne incognita* before nematicide application in 2019 and 2020.

Treatment	Vigor ^a		Stand count		Weight (kg/plot)	Total number of fruits	<i>M.</i> <i>incognita</i> /10 0 cm ³ (before nematicide application)
	28 DAT	56 DAT	28 DAT	56 DAT			
<i>Drip tape</i> ^b							
SDT	0.43 a	0.50 a	12.69 a	8.78 a	11.17 a	178.33 a	2.97 a
SSDT	0.42 a	0.45 a	12.69 a	8.72 a	9.68 a	147.00 a	3.59 a
<i>Nematicide</i> ^c							
Control	0.46 a	0.51 a	13.00 a	9.06 a	13.04 a	193.09 a	1.88 a
Fluensulfone	0.40 a	0.43 b	12.50 a	8.44 a	7.71 a	126.65 a	4.88 a
Fluazaindolizine	0.42 a	0.49 ab	12.75 a	9.13 a	9.93 a	161.50 a	2.50 a
Fluopyram	0.40 a	0.46 ab	12.50 a	8.38 a	11.02 a	169.41 a	3.88 a
<i>P</i> -value							
Year	0.0001	0.0290	0.6255	0.0674	0.3037	0.0453	0.0002
Drip tape	0.7962	0.1614	1.0000	0.8543	0.1452	0.1861	0.9816
Drip tape × year	0.1552	0.5193	0.1639	0.1649	0.0870	0.1448	0.5583
Nematicide	0.0596	0.0390	0.1092	0.1534	0.0979	0.2111	0.1473
Nematicide × year	0.8111	0.5781	0.6254	0.7239	0.3912	0.7918	0.8527
Drip tape × Nematicide	0.5335	0.9038	0.7585	0.1920	0.9725	0.7570	0.4435
Drip tape × nematicide × year	0.8448	0.5455	0.8979	0.8432	0.5742	0.2573	0.1225

DAT (days after transplanting).

^a Vigor rating of the tomato plants were measured with the aid of a handheld crop sensor using a scale of 0 – 1.

^b Data are the means of two years and four replications ($n = 8$).

^c Data are the means of two years and eight replications ($n = 16$).

Means with same letter within each treatment level column (drip tape, nematicide) are not significantly different ($P < 0.05$).

Table 3.2

Effect of treatments on root-knot galling of tomato caused by *Meloidogyne incognita* and the soil population densities of second-stage juveniles of *M. incognita* at midseason and end of the season in 2019 and 2020.

Treatment	Root gall index ^a				<i>M. incognita</i> /100 cm ³			
	At midseason ^b		At end of the season ^c		At midseason ^b		At end of the season ^c	
	2019	2020	2019	2020	2019	2020	2019	2020
<i>Drip tape</i> ^d								
SDT	0.29 a	0.00	1.13 a	0.08 a	29.00 a	1.50 a	41.38 a	1.00 a
SSDT	0.31 a	0.04	1.54 a	0.24 a	22.38 a	1.25 a	50.50 a	4.25 a
<i>Nematicide</i> ^e								
Control	0.92 a	0.00	3.73 a	0.30 a	76.75 a	3.25 a	134.75 a	2.00 a
Fluensulfone	0.00 b	0.00	0.08 c	0.13 a	3.75 ab	0.25 a	1.00 c	2.50 a
Fluazaindolizine	0.13 b	0.00	0.78 bc	0.03 a	2.00 b	0.50 a	16.00 bc	2.50 a
Fluopyram	0.17 b	0.08	0.75 b	0.18 a	20.25 ab	1.50 a	32.00 b	3.50 a
<i>P-value</i>								
Drip tape	0.6391	-	0.1516	0.1121	0.2033	0.5371	0.0874	0.2855
Nematicide	0.0002	-	<0.0001	0.1332	0.0213	0.4106	<0.0001	0.8493
Drip tape × nematicide	0.8092	-	0.1125	0.7805	0.9354	0.1343	0.0981	0.4885

^a Gall rating were done using a gall index of 0 – 5 with 0 being no galls seen on roots and 5 being >75% of root system galled.

^b Midseason = 39 days after transplanting.

^c End of the season = 77 days after transplanting.

^d Data are the means of two years and four replications ($n = 8$).

^e Data are the means of two years and eight replications ($n = 16$).

Means with same letter within each treatment level column (drip tape, nematicide) are not significantly different ($P < 0.05$).

Year data was not combined because there was a year × nematicide interaction for midseason root gall index ($P < 0.0001$), end of season gall index ($P < 0.0001$), end of season soil population density of *M. incognita* ($P < 0.0001$) and year × nematicide × drip tape interaction for midseason soil population density of *M. incognita* ($P = 0.0489$).

Table 3.3

Effect of treatment on the soil population densities of *Paratrichodorus* spp., and *Mesocriconema* spp. in 2019 and 2020.

Treatment	<i>Paratrichodorus</i> count/100 cm ³			<i>Mesocriconema</i> count/100 cm ³		
	Before nematicide application	At midseason ^a	At end of the season ^b	Before nematicide application	At midseason ^a	At end of the season ^b
<i>Drip tape</i> ^c						
SDT	1.50 a	0.56 b	0.56 a	0.13	3.13 a	3.63 a
SSDT	0.75 a	2.88 a	1.13 a	0.06	2.31 a	2.00 a
<i>Nematicide</i> ^d						
Control	1.19 a	2.63 a	0.38 a	0.00	3.25 a	5.63 a
Fluensulfone	1.44 a	1.88 a	0.63 a	0.19	1.50 a	0.88 a
Fluazaindolizine	1.00 a	1.00 a	0.13 a	0.06	3.88 a	1.25 a
Fluopyram	0.88 a	1.38 a	2.25 a	0.13	2.25 a	3.50 a
<i>P</i> -value						
Year	0.0006	0.0460	0.3672	-	0.0195	0.0183
Drip tape	0.2119	0.0056	0.8818	-	0.6924	0.5717
Drip tape × year	0.2119	0.1318	0.0827	-	0.8372	0.5717
Nematicide	0.9478	0.4905	0.0687	-	0.4199	0.1619
Nematicide × year	0.5186	0.8066	0.1761	-	0.2577	0.1619
Drip tape × Nematicide	0.9901	0.0850	0.8948	-	0.0317	0.9852
Drip tape × nematicide × year	0.9901	0.6757	0.4390	-	0.0287	0.9852

^a Midseason = 39 days after transplanting.

^b End of the season = 77 days after transplanting.

^c Data are the means of two years and four replications ($n = 8$).

^d Data are the means of two years and eight replications ($n = 16$).

Means with same letter within each treatment level column (drip tape, nematicide) are not significantly different ($P < 0.05$).

Table 3.4

Effect of treatments on tomato vigor, stand count, fruit yield and root-knot galling of tomato caused by *Meloidogyne incognita* in 2021.

Treatment	Vigor ^a		Stand count		Weight (kg/plot)	Total number of fruits	RGI at the end of the season
	28 DAT	56 DAT	28 DAT	56 DAT			
<i>Drip tape</i> ^b							
SDT	0.04 a	0.24 a	9.10 a	9.05 a	3.99 a	62.88 a	1.92 a
SSDT	0.05 a	0.30 a	8.74 a	8.73 a	3.70 a	60.37 a	2.35 a
<i>Nematicide</i> ^c							
Control	0.05 a	0.29 a	9.33 a	9.43 a	3.74 a	59.78 a	2.98 a
Fluensulfone	0.04 a	0.24 a	8.49 a	8.23 b	4.00 a	60.83 a	1.17 b
Fluazaindolizine	0.05 a	0.29 a	9.00 a	9.15 ab	3.95 a	62.16 a	1.84 b
Fluopyram	0.05 a	0.25 a	8.88 a	8.76 ab	3.68 a	63.74 a	2.55 a
<i>P</i> -value							
Experiment	0.7081	0.2366	0.7438	0.9231	0.6908	0.4318	0.0034
Drip tape	0.2767	0.1417	0.2290	0.3356	0.6071	0.7664	0.1152
Drip tape × experiment	0.1211	0.4784	0.4763	0.2687	0.3285	0.2790	0.5978
Nematicide	0.3373	0.2558	0.1957	0.0566	0.9688	0.9855	<0.0001
Nematicide × experiment	0.7525	0.4968	0.8927	0.9364	0.4863	0.4924	0.0738
Drip tape × Nematicide	0.1417	0.2694	0.4299	0.6116	0.8951	0.9924	0.3954
Drip tape × nematicide × experiment	0.5083	0.4088	0.6505	0.7502	0.5198	0.2904	0.7786

DAT (days after transplanting); RGI (root gall index).

^a Vigor rating of the tomato plants were measured with the aid of a handheld crop sensor using a scale of 0 – 1.

^b Data are the means of two experiments and five replications ($n = 10$).

^c Data are the means of two experiments and ten replications ($n = 20$).

Means with same letter within each treatment level column (drip tape, nematicide) are not significantly different ($P < 0.05$).

Table 3.5

Effect of treatments on the soil population densities of *Meloidogyne incognita*, *Paratrichodorus* spp., and *Mesocriconema* spp. in 2021.

Treatment	<i>M. incognita</i> count/100 cm ³		<i>Paratrichodorus</i> count/100 cm ³		<i>Mesocriconema</i> count/100 cm ³	
	Before nematicide application	At end of the season ^a	Before nematicide application	At end of the season ^a	Before nematicide application	At end of the season ^a
<i>Drip tape</i> ^c						
SDT	13.30 a	424.70 a	0.25 a	0.83 a	15.85 a	5.05 a
SSDT	6.92 b	465.61 a	0.28 a	0.61 a	4.47 a	4.00 a
<i>Nematicide</i> ^d						
Control	14.84 a	562.95 a	0.37 a	0.74 a	13.00 a	5.74 a
Fluensulfone	5.79 a	169.58 b	0.21 a	0.53 a	4.16 a	4.00 a
Fluazaindolizine	4.68 a	449.53 a	0.21 a	0.63 a	10.42 a	4.37 a
Fluopyram	15.79 a	594.26 a	0.26 a	1.00 a	14.26 a	4.11 a
<i>P</i> -value						
Experiment	0.0528	0.0001	0.0545	0.7691	0.5733	0.0570
Drip tape	0.0323	0.2363	0.6941	0.5317	0.0561	0.4802
Drip tape × Experiment	0.9897	0.8089	0.8746	0.5776	0.3239	0.7986
Nematicide	0.3407	< 0.0001	0.2533	0.4411	0.3515	0.8773
Nematicide × experiment	0.2446	0.2636	0.3117	0.8280	0.7476	0.9194
Drip tape × Nematicide	0.7338	0.0944	0.4548	0.0080	0.6641	0.6040
Drip tape × nematicide × experiment	0.1968	0.6858	0.0523	0.2062	0.8578	0.4219

^a End of the season = 77 days after transplanting.

^c Data are the means of two experiments and five replications ($n = 10$).

^d Data are the means of two experiments and ten replications ($n = 20$).

Means with same letter within each treatment level column (drip tape, nematicide) are not significantly different ($P < 0.05$).

CHAPTER 4

CONCLUSIONS

The use of plasticulture for vegetable production is a common growing practice in Georgia and Southeastern United States. This cropping system is faced with the challenge of infestation by RKN that can lead to significant yield loss if not properly managed (Hallmann and Meressa, 2018; Marquez et al., 2021). This research project was aimed at adding new chemical management options and techniques to the toolbox of the growers for the control of RKN. To do this, two independent studies were conducted.

In the first study, we carried out a two-year field trial to investigate the best chemical management options for RKN in a double-cropped vegetable plasticulture system. Usually, growers fumigate while laying the plastic mulch for the first crop and apply nematicides through drip irrigation systems for subsequent crops grown on the plastic mulch (Desaeger and Watson, 2019; Desaeger et al., 2008; Morris et al., 2015; Sanders et al., 1996). The phase-out of methyl bromide and registration of new nematicide products has necessitated research to seek the best chemical choices and practices for proper RKN management and greater crop production. From this study, we observed the mixture of two fumigant products (i.e., 59.6% chloropicrin plus 39% 1,3-dichloropropene) to be a good choice for RKN control and yield increase for the first crop (pepper) grown on plastic beds. In addition, each of the non-fumigant nematicides (fluensulfone, fluazaindolizine, fluopyram, oxamyl, and *Burkholderia* spp. strain A396) examined in the second crop (squash) grown on the plastic mulch was found to be a good option for the RKN control.

In the second study, we sought an optimized technique for non-fumigant nematicides (fluensulfone, fluopyram, fluazaindolizine) application for RKN control in tomato grown on plasticulture by comparing different drip tape application methods in multi-year field trials. The vertical migration of RKN up to depths of 45 cm has been observed (McSorley and Dickson, 1990) and application of nematicides to the surface of the soil around the root zone might not offer adequate control for RKN that are present deep in the soil profile. And these RKN at much lower depth might move upward and cause damage to the plants later in the season (Noling, 2016). In this study, we documented that application of three non-fumigant nematicides at the surface and sub-surface regions of the soil had comparable nematocidal efficacy and yield to only surface application of the nematicides.

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