ABSTRACT

WONG, TSZ WAI SAMMI. Management of Root-knot Nematodes in North Carolina and Sensitivity of Watermelon Pathogens to Succinate Dehydrogenase Inhibitors. (Under the direction of Dr. Lina Quesada-Ocampo).

Root-knot nematodes (*Meloidogyne* spp.) are some of the most economically important and common plant parasitic nematodes in North Carolina cropping systems. These nematodes can be managed using chemical control and cultural methods such as crop rotation. While the southern root-knot nematode, *M. incognita*, has been a large problem in North Carolina, the guava root-knot nematode, *M. enterolobii* has become an emerging threat that is impacting many sweetpotato growers. To understand the incidence and distribution of root-knot nematodes (RKN) in the state, soil samples from fields rotated with sweetpotato were collected from 2015 to 2018 across all counties of North Carolina. Amongst these samples, the highest occurrence of RKN-positive were found in Cumberland, Sampson, and Johnston counties. In addition, Sampson and Nash counties had the highest average RKN population density while Wayne and Greene counties had the lowest average RKN population density. Moreover, we analyzed the host susceptibility of 18 plants for a North Carolina population of *M. enterolobii* by conducting greenhouse trials and measuring the eggs per gram of fresh root (ER) after 45 days. The tomato 'Rutgers' was used as a susceptible control. *M. enterolobii* was able to reproduce on all plants. Two watermelon varieties, cabbage, pepper, one soybean variety, and tobacco were rated as good hosts. Broadleaf signalgrass, corn, one peanut variety, sudangrass, and nutsedge were less susceptible to *M. enterolobii* and considered poor hosts. More research needs to be done to understand the distribution of RKN in North Carolina and the susceptibility of a larger range of crops to ME.

For chemical control of RKNs, newly developed succinate dehydrogenase inhibitors (SDHIs) with potential fungicidal and nematicidal activity provide the opportunity to control multiple diseases with one compound. However, SDHIs have a high risk of fungal pathogen populations developing resistance. If newer SDHIs were to be used to control RKN, its potential effect on fungal populations should be examined. In this study, we aimed to determine the sensitivity of *Meloidogyne incognita* race 4 (MI4), *Fusarium oxysporum* f.sp. *niveum* (FON), and *Stagonosporopsis citrulli* (SCIT), to existing and new SDHIs: benzovindiflupyr, fluopyram, an experimental, and pydiflumetofen. Assays targeting two life stages were conducted for FON and MI4. Cross-sensitivity for SDHIs were also determined for the fungal pathogens. Findings revealed that all SDHIs had fungicidal activity against 19 SCIT isolates, but isolates were most sensitive to pydiflumetofen and the least sensitive to the experimental compound. A positive correlation was seen between pydiflumetofen and all other SDHIs. For FON, we found isolates to be most sensitive to pydiflumetofen and the experimental in the conidial germination assay. However, significant positive correlations between FON isolate sensitivity to the experimental and pydiflumetofen indicate a potential for cross-resistance between these SDHIs. Only fluopyram and the experimental were effective against MI4, particularly in the egg hatch assay. Overall, results suggest that the experimental may be used for managing root-knot nematode, whereas it should be used judiciously for Fusarium wilt of watermelon and gummy stem blight due to the existence of insensitive isolates to the fungicide.

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Management of Root-knot Nematodes in North Carolina and Sensitivity of Watermelon Pathogens to Succinate Dehydrogenase Inhibitors

by
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DEDICATION

To my family,

who helped me in all things great and small.

And to Kegan,

for your steadfast support.

BIOGRAPHY

Tsz Wai "Sammi" Wong was born on May 17th, 1990 in Hong Kong. Her immediate family left Hong Kong in 1992 to Chicago after the British handover of the island. Growing up in the suburbs of Chicago, Sammi was an avid linguist who loved to travel. After leaving for college, she never stayed in one place for more than a few years. She completed her B.A. in French and International Studies at the University of Illinois, Champaign-Urbana, during which, she studied in France and interned at the United States Embassy in Paris. After graduating and studying Turkish in Istanbul, she volunteered with AmeriCorps in Washington State on a community farm, which piqued her interest in agriculture. Sammi then went on to earn a M.S. in International Agricultural Development at UC Davis, where she had the opportunity to work with USAID in Haiti and the International Center for Tropical Agriculture in Colombia. During her time at Davis, she took a course on plant pathology and decided to pursue more technical knowledge in this area. Later, she joined NC State as a M.S. student with Dr. Lina Quesada-Ocampo to research watermelon diseases. While there, Sammi discovered her passions for applied research and bridging the gap between the social and natural sciences.

Outside of work, Sammi enjoys salsa dancing, making succulent arrangements, and anything related to the mystery genre.

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CHAPTER 1

Literature Review

Plant parasitic nematodes

Nematodes are aquatic, unsegmented roundworms found in a wide array of environments that range from marine, freshwater, and soil habitats to multicellular hosts. Regardless of being dependent on water, some nematodes are able to survive in dry environments, where they cease metabolic activity until rehydration (Treonis and Wall 2005). Nematodes are the most numerous Metazoans, and it is estimated that 2-20 million nematodes could be found in one square meter of soil (Decraemer and Hunt 2006). The origin of the word comes from the Greek word 'nematos' meaning thread. Free living nematodes, which consume bacteria, fungi, and protists, are beneficial in the decomposition of organic matter and as biocontrol for other pests. In contrast, parasitic nematodes can injure crops and animals, including and people. Despite their diversity, all nematodes share a basic lifestyle that consists of an egg, four juvenile (J1-J4) stages, and an adult stage. These life stages are separated by four molts of the outer cuticle (Agrios 2005).

Approximately 14% of all known nematode species parasitize plants (Ayoub 1980). A defining structure in all plant parasitic nematodes (PPNs) is the stylet, a protractible hollow needle-like mouth part that can be used for hatching, host invasion, and feeding. In addition, most PPN species have enlarged esophageal gland cells that secrete effectors through the stylet and into the plant (Maule and Curtis 2011). Currently, about 4,100 species PPNs have been described and a majority of them are in the suborder Tylenchina (Jones et al. 2013).

Host plant genotypes, temperature, and soil type are important ecological factors for PPN distribution. During the infective juvenile stage, PPNs must feed on a susceptible host or starve

to death. However, their biology permits the ability of nematodes to enter facultative diapause to survive for long periods of time without a host plant, which adds to the difficulty of management (Chitwood and Perry 2009). Almost all PPNs live part of their lives in the soil, feeding freely from roots in the soil or as sedentary biotrophs within plant roots. A majority of PPN thrive in sandy soils and at higher temperatures, but some species can survive well in heavier soils and colder climates (Mai 1985).

PPNs can cause significant losses in agriculture. In 1987, estimated annual damage in crop loss worldwide due to PPNs was estimated at $70 billion US dollars, which equates to approximately 8-15% crop loss (Kiontke and Fitch 2013; Sasser and Freckman 1987). When adjusted for inflation, this estimate would be a little over $150 billion today. In 1994, the impact in North Carolina was estimated at around $1 billion in tobacco ($1.65 billion in 2017 inflation-adjusted dollars), around $40 million in cucurbit ($66 million adjusted for inflation), and $55 million in sweetpotato ($90 million adjusted for inflation) (Koenning et al. 1999). In addition, associations of PPN with other plant pathogens can account for further damage.

Root-knot nematodes

Some of the most economically damaging PPNs are from the genus *Meloidogyne,* commonly known as the root-knot nematodes (RKN). The name “root-knot” comes from the characteristic intercalary galls or knots that form on infected roots. All RKN penetrate plant roots and transform selected root vascular cells into feeding sites called “giant cells” that exhibit extreme hyperplasia, an increase in cell number, as well as multiple nuclei and dense cytoplasm (Moens et al. 2009). Hyperplasia, an increase in cell size, is induced around the giant-cells and results in the characteristic root galling symptom (Moens et al. 2009). RKN usually go through

several (~30 days) life cycles during a typical growing season and are hatched juveniles are able to re-infect roots multiple times, increasing root damage. Towards the end of a growing season, plants can develop root necrosis and rot. Above ground symptoms are similar to water and nutrient deficiency such as chlorosis, wilting, and stunting (Moens et al. 2009). Due to the inconspicuous symptoms, it is often difficult to assess nematode damage in crop production, especially in countries where diagnoses of nematode infection are limited or absent.

The majority of RKNs can be found 5-30 cm beneath the soil surface and the population decreases in lower soil depths. However, this is highly dependent on the root system of the host. For example, nematodes have been found as deep as 5m in perennial systems (Taylor et al. 1982). On average, nematodes are capable of migrating as fast as 25cm vertically and 50cm horizontally in 10 days (Prot 1980). Since they are unable to move quickly through a field, they are often found in clusters, or foci, in the field (Ferris and Noling 1987).

RKN is a cosmopolitan pathogen with species that cover a wide host range of more than 3,000 plant species (Abad et al. 2003). In 1976, a global survey of PPNs found the most economically important and abundant species to be *M. incognita* (Southern root-knot nematode), *M. javanica* (Javanese root-knot nematode), *M. arenaria* (Peanut root-knot nematode), *M. hapla* (Northern root-knot nematode), and it was estimated that these four species were accountable for approximately 5% of annual global crop loss (Sasser et al. 1983). These four RKN spcies can be categorized into thermophils and cryophils (Curtis et al. 2009), which describe how these organisms behave in different temperatures. Cryophils like *M. hapla* are able to hatch and survive in soil temperature below 10°C and are found in colder regions. Thermophils like *M.incognita*, *M. javanica*, and *M. arenaria* cannot hatch below 15°C (Curtis et al. 2009). Out of all four species, *M. incognita* is considered to be the most damaging since it is most abundant

and widespread (Mai 1985). Host differential tests of *M. incognita* showed four different races, with race 4 being the most pathogenic (Swanson and Van Gundy 1984). In addition, host susceptibility may differ depending on the species of RKN.

Emerging threat of guava root-knot nematode

In recent years, a highly aggressive tropical root-knot nematode species, *M. enterolobii* (syn. *M. mayaguensis*), has become an emerging threat in many countries (Castagnone-Sereno 2012). *M. enterolobii*, known as the guava root-knot nematode, was originally found in southern China on the pacara earpod tree (*Enterolobium contortisiliquum*) (Yang and Eisenback 1983). In the US, it was originally reported in Florida, but has since been detected in North Carolina (Brito et al. 2004; Ye et al. 2013) and South Carolina (Rutter et al. 2018). Recently, a quarantine on contaminated sweetpotato seeds and plants was enacted after discovering this species in 8 counties of North Carolina (Ye 2018). *M. enterolobii* is of particular concern since it has a similar host range to *M. incognita*, but has been able to reproduce on crops with resistance against major *Meloidogyne*. spp. (Castagnone-Sereno 2012).

Life cycle of root-knot nematodes

Eggs are deposited in a gelatinous matrix (egg sac) on the root gall surface by females embedded within the plant root. RKNs are sedentary and endoparasitic meaning they spend the majority of their life cycle inside the host plant root. After embryogenesis, the first molt occurs within the egg and the infective J2 (second juvenile stage) emerges from the egg in the soil. The hatching of J2 is highly dependent on favorable environmental conditions, particularly at soil temperatures above to 12°C and optimal soil moisture levels (Goodell and Ferris 1989). After

hatching, the J2 migrates in soil towards a root and must rely on stored energy reserves until penetrating a host plant root. The J2 penetrates the root near the zone of elongation behind the root tip and moves up the vascular cylinder after reaching the meristematic zone at the tip (Abad et al. 2009). The infective J2 injects effectors using its stylet into selected root vascular cells around its head that will become multinucleated giant cells. Once giant-cells are formed, the RKN commences feeding from them and swells to become a sedentary parasite. The feed site acts as a nutrient sink, which facilitates the extraction of nutrients and fluids through cell wall ingrowths adjacent to the xylem (Bird 1961) and upregulate plant metabolic genes (Abad et al. 2009). Plant growth regulators such as auxins and cytokinins have been linked to the development of giant cells and galls (Lohar et al. 2004). Under ideal temperatures and host, *M. incognita* can complete its life cycle in as little as 20 days (Ploeg and Maris 1999)

RKNs are primarily parthenogenic, meaning males are not necessary for reproduction, but some species are capable of sexual reproduction (Chitwood and Perry 2009). The sex of RKN is determined by genotype and influenced by environmental factors during the J2-J3 stage (Triantaphyllou 1973) Under environmentally favorable conditions such as food availability and optimal temperature, fourth-stage juveniles (J4) become female and continue to be sedentary and swell up into a pyriform shape (Davide and Triantaphyllou 1967). Unfavorable conditions such as heavy host infection and sub-optimal temperatures can lead to higher male populations (McSorley 2003). This ability is a survival mechanism for nematodes since males can leave the root to reduce the parasitic load and the progeny are essentially clones of the female that can quickly adapt to a new environment.

Management of root-knot nematodes

Nematodes are extremely difficult to manage and or eradicate once they are established in the field. RKNs have a high reproductive rate and are able to go through several lifecycles during a growing season. Sampling of soil and roots is an important aspect of RKN management to determine if action should be taken since crop damage is generally related to the initial nematode population density. In addition to forecasting nematode crop loss, sampling is important in diagnosing existing nematode problems and informing growers on appropriate management strategies (Barker and Nusbaum 1971). Predictive samples are taken in the fall after harvest and diagnostic samples during the growing season. It is often recommended to submit samples for soil nutrient analysis since nematode and soil fertility symptoms are similar. There are action thresholds for different crops depending on which species of nematodes are found in the field (Ye 2015).

Sampling is an important aspect of In addition to forecasting nematode crop loss, sampling is important in diagnosing existing nematode problems and informing growers on appropriate management strategies (Barker and Nusbaum 1971). It is often recommended to submit samples for soil nutrient analysis since nematode and soil fertility symptoms are similar. There are action thresholds for different crops depending on which species of nematodes are found in the field (Ye 2015).

Several cultural methods are used to prevent the spread of nematodes and reduce populations in the field. Sanitizing farm equipment and preventing the movement of contaminated soil or planting materials can limit the spread of nematodes to new areas. Crop rotation is a viable option if the nematode species in the field does not have a broad host range. Antagonistic crops like marigolds (*Tagetes* spp.) have been shown to reduce populations of

Meloidogyne spp. due to alphaterthienyl, a toxic compound produced in the roots (Hallmann et al. 2009). This is often not economically practical for farmers if the non-host crop is of lesser value or the farmer needs to purchase specialized equipment for its harvest. Other methods focus on manipulating the soil environment such as soil solarization, flooding, fallowing, and the addition of soil amendments (Oka et al. 2007). However, heat treatments like solarization are only effective against nematodes in the upper layer of soil. Soil flooding and fallowing can starve the nematode populations, but requires the land to be out of production which is not practical for the farmer (Viaene et al. 2006). The addition of organic soil amendments such as chitin, compost, and plant residues can lower existing PPN populations in the field (Oka 2010).

The use of biological control agents (BCAs) such as microbial parasites, predators, and antagonists have also been researched for management. Bacteria and fungi are the most promising biocontrol agents, but their efficacy is often affected by abiotic facts such as soil moisture and pH (Stirling 2011; Jatala 1986). For example, the bacteria *Pasteuria penetrans*, associated with nematode-suppressive soils, produces endospores that parasitize RKNs and lowers reproduction rates of females (Viaene et al. 2006). Other rhizosphere bacteria such as *Bacillus* spp., antagonize nematodes through the production of toxins, enzymes, and secondary metabolites. Nematophagous fungal parasites and predacious nematode-trapping fungi have also been analyzed with varying success as methods of control (Viaene et al. 2006). *Burkholderia* spp., a bacteria used for nematode biocontrol, is commercially available and approved for organic vegetable production in North Carolina (NCDA 2016).

Host resistance against RKN is an integral part of management. Single gene resistance has been incorporated into a number of crop cultivars (Williamson and Roberts 2009). Induced host resistance occurs when a single gene present in the host plant recognizes nematode

avirulence gene products such as effectors (Haegeman et al. 2012). Most nematode resistance genes target the post-infection phase (Williamson and Roberts 2009). For example, resistant cultivars induce a hypersensitive reaction (HR) or cell death in the root, which prevent juveniles from a forming a functional feeding site and develop into adults. RKN-resistant cultivars support low nematode reproduction and typically display reduced galling. (Williamson and Roberts 2009). The best-characterized nematode resistance gene is the Mi-1 gene in tomato that conferred resistance against *M. incognita*, *M. javanica*, and *M. arenaria* for over 40 years. However, it has been discovered that the resistance gene is less effective at high soil temperatures and nematode populations have been able to overcome Mi-1 resistance in areas of high tomato production (Castagnone-Sereno 2002). Another method for utilizing resistance is through grafting, which utilizes rootstock that has some resistance or tolerance to nematodes (Thies et al. 2010). As with other pathogens, the development of resistance-breaking races within target species of RKN has been reported (Williamson and Roberts 2009; Castagnone-Sereno 2012), and demonstrates the importance of integrated approach to nematode management.

Chemical control has been used since the late 19th century and can be the predominant means for nematode management in crops that provide economic returns that exceed the cost of nematicide application. Nematicides are often used in conjunction with cultural methods and host resistance as an integrated pest management strategy. Estimated nematicide use in the global market is $1 billion USD of which 48% of applications is allocated towards control of RKNs (Haydock et al. 2013). Nematicides can be classified into two groups, fumigants and non-fumigants. Some non-fumigant compounds, such as the organophosphates and carbamates, can be described as “nematistatic” as they do not directly kill, but cause a paralysis that can be reversed under conditions below the compound’s effective (Opperman and Chang 1992).

Throughout this thesis, the term "nematicides" will be used to describe any chemical used for nematode control.

Fumigants are applied directly in the soil where they volatize and disperse through the soil as a gas. They are often applied before planting since fumigants are phytotoxic. Fumigants are highly effective in controlling PPNs; however, they can be expensive since application requires specialized equipment to inject the product deep in the soil and some require plastic tarps to prevent volatilization into the environment (Haydock et al. 2013). The first fumigant shown to be effective against PPN was a carbon disulfide and was followed by the development of other fumigants such as halogenated hydrocarbons and methyl isothiocynate liberators (Chitwood 2003). Halogenated carbons, which includes 1,3-dichloropropene (1,3-D) and methyl bromide, are believed to target protein synthesis and respiration. Methyl isothiocynate liberators such as metam sodium break down to release cyanide, which prevents cellular respiration (Chitwood 2003). Fumigants are highly effective because they distribute broadly in the soil profile and sometimes target a broad spectrum of soilborne pathogens and pests (Haydock et al. 2013). One of the more popular nematicides still widely used in the US is 1,3-D (Telone II), but it has been banned in the EU due to non-target toxicity (Nyczepir and Thomas 2009). Several fumigants have also been withdrawn due to concerns over environmental and human health. Dibromochloropropane (DBCP) was deregistered in the 1970's for causing male sterility in workers manufacturing the chemical and ethylene dibromide (EDB) was withdrawn from the market during the early 1980s due to groundwater contamination and carcinogenic effects (Chitwood 2003). Moreover, methyl bromide, a highly effective broad-spectrum fumigant, has been identified as a factor in ozone depletion and banned in many countries (Zasada et al. 2010)

First-generation non-fumigant (contact) nematicides were derived during the 1960's from organophosphates and carbamates insecticides that cause paralysis by inhibiting acetylcholinesterase (AChE), an enzyme essential for neurotransmission (Haydock et al. 2013). Unlike fumigants, contact nematicides are non-volatile and cannot permeate throughout the soil as effectively tobacco (Nyczepir and Thomas 2009). They are mainly applied as liquid or granular formulation to soil and occasionally as a systemic foliar spray (Oka et al. 2012). The paralysis caused by AChE inhibitors can deplete nematode lipid reserves to the extent that they are unable to parasitize the plant (Chitwood 2003). In addition, some non-fumigant nematicides such as aldicarb, fenamiphos, and oxamyl are highly mobile in soil and able to leach into groundwater, increasing the likelihood of damage to nearby ecosystems (Bilkert and Rao 1985). Currently, aldicarb (Temik) has been re-introduced to market as AgLogic in a different formulation and under stricter regulations.

Succinate Dehydrogenase Inhibitors

Potential toxicity to non-target organisms and environmental concerns have led to severe restrictions in labeling and use of many early generation fumigant and non-fumigant nematicides (Chitwood 2003). While a few nematicides remain labeled for use in specific crops, many of the original and cost-effective nematicides are no longer available or affordable. Safer and more cost-effective nematicides are in demand and this has prompted the development of a new generation of active ingredients (Nyczepir and Thomas 2009). One such example are some succinate dehydrogenase inhibitors (SDHIs), which were developed as fungicides and found to also have nematicidal properties against *M. incognita* (Faske and Hurd 2015; Heiken 2017).

Succinate dehydrogenase (SDH) is an enzyme complex bound to the inner mitochondrial membrane (McKay et al. 2011). SDHIs are compounds that block the ubiquinone-binding sites in complex II of the mitochondria, which effectively inhibits fungal respiration (Avenot and Michailides 2010). When a compound is bound to complex II, it displaces ubiquinone and suppresses the completion of the electron transport chain to produce ATP (McKay et al. 2011).

In the late 1960s, the first generation of SDHIs was carboxin and was used as a narrow spectrum fungicide against basidiomycete pathogens (Sierotzki and Scalliet 2013). A variety of new SDHI fungicides such as benodanil, fenfuram, and flutolanil were released in subsequent years, but were mostly effective against basidiomycetes and not ascomycetes. The first truly broad-spectrum SDHI was boscalid, a pyridine carboxamide able to control ascomycete fungi such as *Botrytis* spp., *Sclerotinia* spp., and *Alternaria* spp. (Avenot and Michailides 2010). The Fungicide Resistance Action Committee (FRAC) has grouped SDHIs of various chemical subclasses into FRAC group 7, a cross-resistance group that means resistance to one fungicide will confer resistance to other fungicides within this class.

Resistance is a concern with SDHIs since they bind to a single site in the target organism. SDHI resistance can develop when the ubiquinone-binding fungal target site mutates, thereby reducing fungicide sensitivity (Sierotzki and Scalliet 2013).. After the increased usage of foliar SDHI fungicides, several cases of resistance have been reported in: *Botrytis cinerea*, *Alternaria alternata*, and *Didymella bryoniae* (Amiri et al. 2014; Avenot et al. 2014, 2012). Cross-sensitivity of SDHI fungicides is complex because studies have shown that fungal mutations confer full cross-resistance to some SDHIs and not to others. For example, a study on *A. solani* showed that isolates resistant to boscalid were not resistant to fluopyram, a SDHI of a different chemical subclass (Fairchild et al. 2013). However, cross-resistance of fluopyram and

penthiopyrad has been reported in *Botrytis. cinerea* and *Venturia inaequalis* (Amiri et al. 2014; Villani et al. 2016). The eventual development of SDHI resistance and differences of SDHI cross-resistance profiles highlights the importance of monitoring pathogen populations over time on new and existing SDHIs. Resistance can be managed by applying different fungicides simultaneously or rotating fungicides of different modes of action (McKay et al. 2011).

Watermelon production, an ideal system to evaluate SDHIs

Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae, which also includes other important vegetables such as cucumber, pumpkin, gourds, and other types of melon. The US is one of the top ten producers and consumers of watermelon with North Carolina being the 5th largest producer (FAOSTAT 2017; USDA-NASS 2019). Most watermelon production in the US occurs in the south due to the warm environment and sandy soils, which are ideal growing conditions for this crop (Holmes et al. 2005). Crops such as watermelon are commonly affected by different pathogens during a growing season (Keinath et al. 2017); thus, having products that can control several diseases is desirable.

Important nematode and fungal watermelon pathogens include RKN, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON), and gummy stem blight (GSB) caused by caused by *Stagonosporopsis citrulli* (SCIT) (syn. *Didymella bryoniae*), *S. cucurbitacearum,* and *S. caricae*. New fungicide chemistries in the SDHI class have been recently registered or are pending registration for disease control for these diseases. These new active ingredients (a.i) include benzovindiflupyr, fluopyram, pydiflumetofen, and an experimental compound (under development and pending registration). While fluopyram has been shown to have an effect on

RKNs (Faske and Hurd 2015), understanding the sensitivity of SCIT and FON populations to these new SDHIs is important to avoid resistance development.

Root-knot nematodes are a threat to worldwide and US watermelon production because most cultivars are highly susceptible (Thies and Levi 2007a). The four main species of RKN and the four races of *M. incognita* are able to infect watermelon (Boyhan et al. 2003). A global survey funded by USAID found that 18-33% crop loss of cucurbits in the tropics is due to nematodes (Sasser 1979) and regional studies have shown watermelon crop losses to be as high as 50% (Lamberti 1979). Flupopyram is labeled as a nematicide and broad-spectrum fungicide under the trade name Velum in the United States. It is currently registered in all states except Alaska (Velum Prime 2017). Research has shown fluopyram to provide more control against *Meloidogyne incognita* and *Rotylenchulus reniformis* than other SDHI fungicides (Faske and Hurd 2015).

FON is a soil-borne fungal pathogen that infects and blocks the xylem of watermelons, causing a characteristic wilt symptom (Keinath et al. 2017). Triploid (seedless) watermelon cultivars are susceptible to all 4 races (0, 1, 2, 3) of FON (Zhou et al. 2009). Fusarium is an ascomycete fungus that can produce three spore types: macroconidia, microconidia, and conidiophores. In addition, the fungus produces a survival structure called a chlamydospore that can survive in the soil for up to 10-15 years (Egel and Martyn 2013). Cultural management strategies for FON include planting resistant cultivars, soil solarization, and organic amendments. There are currently no SDHI fungicides labeled to treat FON. In fact, few chemical controls exist for this disease; only prothioconazole, an ergosterol demethylation inhibitor, is currently registered in North Carolina to treat Fusarium wilt in cucurbits (Miller et al.in press). Recently, Miller et al. (in press) found North Carolina FON isolates to be sensitive to

pydiflumetofen, which has been registered for watermelon as Miravis Prime. *Fusarium oxysporum* spp. affects a wide variety of crops , but is host specific, which means FON only infects watermelon (Michielse and Rep 2009). In addition, one of the most researched disease complexes is RKN and *Fusarium oxysporum* spp.. Studies in cotton, tobacco, and tomato have shown increased disease incidence when both *Fusarium oxysporum* spp. and RKNs are present (Mai and Abawi 1987). Fusarium wilt symptoms in cotton were less severe in wilt-resistant cultivars than susceptible ones when there is a presence of nematodes. However, nematode reproduction was similar across both types of cultivars (Manzanilla-López and Starr 2009). In the case of watermelon, RKN infection enhanced susceptibility of watermelon to FON even if watermelon lines show FON resistance or tolerance (Sumner and Johnson 1973).

GSB, a disease caused by the ascomycete fungus SCIT, is very destructive in transplant greenhouses (Koike 1997) and major watermelon-producing areas of the Southeastern US (Keinath 2000). SCIT is a foliar pathogen able to infect all above-ground parts of the watermelon and can result in an average yield loss of 43% in non-treated plots (Keinath and Duthie 1998). Typical symptoms on watermelon include lesions on the leaf and petioles, stem canker, and extensive defoliation. GSB can also result in fruit lesions, but fruit infection is not commonly observed in watermelon. The fungus can overwinter on plant debris, and deep-turning the debris after harvesting is a cultural practice to manage GSB (Keinath 2013). The most effective management option is chemical control because there are no GSB-resistant watermelon cultivars commercially available (Avenot et al. 2012). However, SCIT was able to develop resistance to several single-site fungicides (Avenot et al. 2012). Isolates resistant to the SDHI boscalid (Thomas, Langston, and Stevenson 2012) and the quinone-outside-inhibiting (Qoi) class fungicide azoxystrobin (Keinath 2009) have been reported.

Thesis objectives

This thesis has two main objectives that address cultural methods and chemical control of root-knot nematodes as well as chemical control of fungal pathogens found on watermelon. With the emerging threat of *M. enterolobii* on sweetpotato, this thesis aimed to explore the population distribution of *Meloidogyne* spp. in North Carolina and the host range of *M. enterolobii* on crops and weeds. The distribution, occurrence, and density levels of RKN were derived from previous analysis of soil samples submitted to the Nematode Assay Division of the North Carolina Department of Agriculture from 2014-2018. In addition, we investigated the sensitivity of FON, SCIT, and *M. incognita* race 4 to new SDHI chemistries as well as potential cross-sensitivity between theses SDHIs for the fungal pathogens. The sensitivity of different life stages to the new SDHIs benzovindiflupyr, fluopyram, pydiflumetofen, and an experimental compound were also explored for FON and *M. incognita* race 4.

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CHAPTER 2

Occurrence and distribution of *Meloidogyne* spp. from fields rotated with sweetpotato and host range evaluation of *Meloidogyne enterolobii* in North Carolina

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ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are some of the most economically important and common plant parasitic nematodes in North Carolina cropping systems. Soil samples from fields rotated with sweetpotato were collected from 2015-2018 across all counties of North Carolina (n=39), and incidence and distribution of root-knot nematode (RKN) populations was analyzed. Cumberland (53%), Sampson (48%), and Johnston (48%) counties had the highest number of RKN positive samples. Sampson (147 J2/sample) and Nash (135 J2/sample) counties had the highest average population density while Wayne (7.3 RKN per sample) and Greene (11.4 RKN per sample) counties had the lowest average RKN population density. *M. enterolobii* is a new invasive species that is impacting sweetpotato growers of North Carolina. The host susceptibility of 18 plants, which included important vegetable, field, cover crops, as well as weeds for a North Carolina population of ME, was determined in this study by calculating the eggs per gram of fresh root (ER) values in 4 experiments. The tomato 'Rutgers' was used a susceptible control.

Cabbage (ER = 3,330 and 155), pepper (ER = 64 and 12,810), the watermelon ‘Charleston gray’ (ER = 67 and 1,579) and ‘Fascination (ER = 11,411 and 18,717) were susceptible and had similar ER values to tomato, the positive control included in each experiment. Among field crops, cotton, soybean ‘P5018RX’, and tobacco were suitable hosts with ER values that ranged from 185 to 706. While *M. enterolobii* was able to reproduce on field crops, their ER values were lower than the vegetable crops. Members of the Poaceae family such as sweet corn and sudangrass were the least susceptible to *M. enterolobii* and the average ER values by experiment ranged from 1.85 to 7. The peanut ‘Tifguard’ (ER = 6 and 31) and winter wheat (ER =2 and 39) also had lower ER values than the vegetable hosts. Growers should use less susceptible hosts such as the peanut ‘Tifguard’, sudangrass, sweet corn, and winter wheat in crop rotations to lower populations of this invasive nematode.

INTRODUCTION

Sweetpotato production in the United States (US) has steadily risen since 2000 with Europe and Canada as the main importers (Johnson et al. 2015). However, production can often be limited by many pests and diseases, particularly nematodes. Root-knot nematode (*Meloidogyne* spp.) along with the reniform nematode (*Rotylenchulus reniformis*) are the primary nematode species affecting sweetpotato production (Thomas and Clark 1983). The impact of nematodes on the North Carolina sweetpotato industry was estimated to be $55 million ($94 million adjusted for inflation) (Koenning et al. 1999).

Root-knot nematodes are cosmopolitan and one of the most economically important plant-parasitic nematodes with a wide combined host range of over 3,000 plant species (Abad et al. 2003). Symptoms associated with RKN infection include root galling, chlorosis, and stunted growth. In addition, nematode damage can lead to secondary infections by other pathogens (Manzanilla-López and Starr 2009). Currently, eleven *Meloidogyne* species have been recorded in North Carolina with most plant damage associated with the four main species: *M. incognita, M. javanica*, *M. arenaria*, and *M. hapla* (Ye 2018). Among those species, several RKNs are associated with sweetpotato: *M. incognita*, *M. javanica*, *M. hapla*, and *M. enterolobii* (syn. *M. mayaguensis*) (Jones and Dukes 1980; Karuri et al. 2017). Root-knot nematodes are extremely difficult to manage and are considered to be the most important nematode pathogen in sweetpotato production (Overstreet 2009). The action threshold level for RKN juveniles in sweetpotatoes is >100 per 500 cm^3 of soil (Ye 2015), however any level may result in storage root quality concerns by the end of the growing season (Smith et al. 2017). The galls produced by the nematode disfigures the sweetpotato, making the sweetpotato unmarketable (Ye 2018).

The impact of RKNs on the sweetpotato industry became extremely important since North Carolina, Louisiana, Mississippi, and Arkansas enacted the quarantines on the guava root-knot nematode (*M. enterolobii*). *M. enterolobii* is regarded as a highly aggressive species that induces bigger and higher number of galls than other RKN species (Castagnone-Sereno 2012). *M. enterolobii* was originally thought to be restricted to tropical countries; however, it has been found recently in the Mediterranean and subtropical regions, which has prompted its quarantine by many countries and regulatory agencies (Elling 2013). In the US, *M. enterolobii* was first reported by Brito et al. (2004) from Florida and later in North Carolina (Ye et al. 2013). Recently, a quarantine was placed to prevent the movement of contaminated sweetpotato seed and plants after *M. enterolobii* was detected in eight counties in North Carolina including Columbus, Greene, Harnett, Johnston, Nash, Sampson, Wayne, and Wilson counties as of September 2018 (Koger 2018; Ye 2018).

Current management practices to prevent the spread and reduce populations of RKNs include chemical and cultural controls. Some nematicides, fumigants and non-fumigants, were effective at controlling *M. enterolobii*. Recent sweetpotato field trials showed that Telone II (Corteva Agriscience, Indianapolis, IN) and Velum Prime (Bayer Cropscience, Research Triangle Park, NC) were able to reduce *M. enterolobii* and *M. incognita* damage on sweetpotato (Collins et al. 2018).

The best-characterized nematode resistance gene is the *Mi-1* gene in tomato that conferred resistance against *M. incognita*, *M. javanica*, and *M. arenaria* for over 40 years (Castagnone-Sereno 2002). While its host range is similar to *M. incognita*, *M. enterolobii* has the ability to reproduce on crops carrying major resistance genes effective to most *Meloidogyne* spp. such as resistant sweetpotato (Castagnone-Sereno 2012). Rutter et al. (2018) found *M.*

enterolobii was able to reproduce as effectively on the *M. incognita*-resistant cultivar 'Covington' on *M. incognita*-susceptible cultivar 'Beauregard'. Several studies have been conducted on the host range of *M. enterolobii* in Cuba (Rodriguez et al. 2003), Florida (Brito et al. 2004), and Brazil (Freitas et al. 2017). However, a study has not been done for a North Carolina population of *M. enterolobii* on local crop cultivars and weeds.

The North Carolina Department of Agriculture and Consumer Services' (NCDA) Agronomic division is one of the largest nematode assay labs in the US and has been providing nematode assay services since 1974 (Ye 2018). Farmers submit soil samples each year and the lab provides nematode counts and recommended actions based on the hazard index specific for that crop (Ye 2014). Farmers can submit either predictive soil samples, which are taken in the fall after harvest, or diagnostic samples during the growing season (Ye 2014). Geographic information system (GIS) software has been used successfully to identify counties in the US and Canada to map the location of soybean cyst nematodes (Tylka and Marett 2017; Ye 2017). A geospatial analysis on the distribution of RKNs in North Carolina sweetpotato fields would be highly beneficial to growers since it could guide extension efforts focusing on nematode scouting and management. Improved understanding of potential *M. enterolobii* hosts and RKN occurrence and population density in fields of crops planted prior to sweetpotato would inform a crop rotation strategy for growers. The objectives of this study were to: 1) determine the distribution of *Meloidogyne* spp. found in fields rotated with sweetpotato in North Carolina, 2) assess the occurrence, abundance, and projected risk for growing sweetpotato by previous crop for *Meloidogyne* spp., and 3) understand the host range of *M. enterolobii* among commonly grown crops and weeds found in North Carolina using a local population.

MATERIALS AND METHODS

Distribution of root-knot nematodes in sweetpotato fields in North Carolina

Nematode soil samples from sweetpotato fields submitted to the NCDA were analyzed by the nematode assay lab from 2015-2018. Only soil samples that indicated sweetpotato as a previous or future crop were included in the analyses described below. Following the guidelines from the NCDA, growers collected at least 20 soil samples to a depth of 4-8 inches. The cores were mixed thoroughly and a sample (roughly 500 cm^3 of soil) of that mixture was submitted to the nematode assay lab at the NCDA. Depending on the size of the field and problem areas, growers submitted one or more samples. Soil samples were kept in at 4°C until nematode extraction. Nematodes were extracted from 500 cm^3 of soil using an elutriator and sugar centrifugal floatation (Jenkins 1964; Byrd et al. 1976). Root-knot nematodes were previously identified to the genus level (*Meloidogyne* spp.) by NCDA staff based on morphology of the second-stage juveniles (Eisenback 1985). Distribution maps were created using R 3.6.1 software (R Core Team 2019) and the ggmap package (Kahle and Wickham 2013) from nematode counts recorded by the nematode assay lab. Only predictive samples were considered since they were taken after harvesting the prior crop and samples from research stations were disregarded.

The occurrence (presence/total sample) and average count of RKN per sample was calculated for previous crop for more than 200 samples processed from 2015-2018. In addition, a recommended action was given to each soil sample by the nematode assay lab based on nematode count thresholds for the next crop, county, and previous host (Ye 2015). The projected risk for growing sweetpotato by the RKN counts from the previous crop was considered. Only RKN and the reniform nematode have established threshold levels for sweetpotato. For RKN, a recommendation of A (no action) was given for 0-9 RKN juveniles per 500 cm^3 soil sample, B

(marginal level) was given for 10-99 RKN juveniles per sample, and C (action required) was given for samples with 100+ RKN juveniles per sample (Ye 2015). In terms of management, action recommended usually suggests chemical fumigation, resistant variety, or crop rotation. To estimate the random effects of year, processing date, county, and previous crop on the variability of the average count of RKN found in samples, a generalized linear mixed model was used to fit the data. Samples were analyzed in R 3.6.1 (R Core Team 2019) and the lme4 (Bates et al. 2014) package was used. The average count data per sample was heavily skewed left and a negative binomial distribution was used for the model as a large number of samples did not have RKN.

Host range of *Meloidogyne enterolobii*

This experiment was conducted in the greenhouse at North Carolina State University, Raleigh, NC. The population of *M. enterolobii* tested was from an established culture collected from a soybean field in North Carolina that had been rotated from sweetpotato. Species diagnostic PCR using RKN female DNA extractions and the specific primers Me-F and Me-R was used to verify the species *as M. enterolobii* (Baidoo et al. 2016) (Baidoo et al. 2016). *M. enterolobii* was maintained in greenhouse culture on roots of 'Rutgers' tomato plants and eggs used for inoculations of test plants were harvested from galled tomato roots for 1 minute in 0.5% NaOCl (Hussey 1973) and collected on a sieve with 25-micron openings.

Eighteen different plant species and cultivars were tested against *M. enterolobii* and planted at separate times to ensure development of the 2nd true leaf at the same time (Table 2.1). Seeds were started in 72 cell trays with a 1:1 autoclaved sand to Sunshine® potting mix (Sun Gro Horticulture, Agawam, MA). After reaching the 2nd true leaf stage, plugs were transplanted to 90mm diameter clay pots with a 1:1 autoclaved sand to soil mixture and a gram of 10-10-10

slow release fertilizer was added to the soil surface. Due to limited greenhouse space, the host range test was conducted in batches with 9-12 plant genotypes per trial. Each trial included 'Rutgers' tomatoes as a susceptible control and two runs were conducted for each genotype. The experiment was set up as a complete randomized block design with five replicates of each treatment (genotype). Also, two additional pots without nematodes were used as controls for each treatment. A total of four greenhouse experiments were completed that tested each plant genotype twice. Greenhouse temperatures fluctuated between 27-30 °C during the experiment plants were watered once a day. One week after transplanting, the soil containing roots of each test plant was inoculated with 4,000 eggs 4 ml. of *M. enterolobii*. Water was applied to the control non-treated pots. Plants were removed from pots at 45 days after inoculation and *M. enterolobii* eggs were harvested from inoculated roots using the method mentioned above. Fresh root weight was taken from both the inoculated plants and the non-treated pots were checked for galling. The eggs per gram of root (ER) was determined by dividing total eggs/root system by the total fresh root weight per plant. Nematode reproduction (ER) was used to assess the potential host status of each test plant genotype. All data were analyzed in R 3.6.1 (R Core Team 2019). The ER and transformed log (x+1) ER values generated from each experiment were tested for normality using the Shapiro-Wilk test and data were not normal. Thus, the MASS package (Venables and Ripley 2002) was used to fit ER values from each experiment to a negative binomial GLM. Afterwards, a post hoc Tukey's HSD comparing the estimated marginal means of ER values among the hosts for each experiment was done using the emmeans package (Russell 2018).

RESULTS

Distribution of root-knot nematodes in sweetpotato fields in North Carolina

A total of 55,127 predictive soil samples were processed by NCDA from 2015 to 2018. The number of samples ranged from 12,837 to 15,309 per year with most samples being processed in 2017 and the least number of samples processed in 2016 (Figure 2.1a). Predictive samples were mainly processed during February, March, and April, while the fewest number of samples were processed during June, July, August, and September (Figure 2.1b). However, amongst samples, the percentage occurrence of RKN in processed samples was highest during the fall and early winter months of September, October, and November (Figure 2.1c). While soil samples from fields rotated with sweetpotatoes were submitted from 39 counties (Figure 2.2a) from 2014-2018, only predictive samples from counties (n=15) with more than 100 soil samples were included in the maps for of RKN occurrence (Figure 2.2b) and abundance (Figure 2.2c). The range of RKN from positive samples ranged from 10 to 22,080 RKN per sample. RKN count ranged from 10 to Counties with more than 200 predictive samples were considered for further analysis (n=12). Nash, Edgecombe, Wilson, Johnston, Sampson, Wayne, Duplin, Pitt, Harnett, Greene, Halifax, and Cumberland are located in the eastern part of North Carolina, where a majority of sweetpotato is grown. Of the 12 counties, 8 counties had positive reports of *M. enterolobii*, which included Greene, Harnett, Johnston, Nash, Sampson, Wayne, Columbus, and Wilson (Figure 2.2d)

Counties with more than 3,000 samples submitted included Sampson, Johnston, Wilson, Edgecombe, and Nash. At least 21 to 40 growers or consultants submitted samples from these counties. Among those counties, the occurrence of RKN ranged from 37% to 46% of all predictive samples submitted. Counties with the highest average, ranging from 126 to 147 RKN

juveniles per 500 cm^3soil sample, were Johnston, Wilson, Nash, and Sampson county. Edgecombe had a mean of 68 RKN per sample. Wayne and Duplin had 1,000 to 2,000 submitted samples and had 16 and 14 grower or consultants submit samples. Duplin had the higher percentage occurrence of RKN at 36% as well as higher mean of 34 RKN per sample. Wayne had the lowest percentage occurrence with 10% of samples testing positive and the lowest mean count of 7 RKN per sample. Pitt, Harnett, Greene, Halifax and Cumberland had between 200 to 1,000 samples submitted over the course of four years. Cumberland had the lowest number of individuals (n=3) and of samples submitted (n = 259), but reported the highest percentage of 53% incidence of RKN. The rest of the counties in this group had 7-10 growers or consultants submit samples. Pitt and Harnett had similar percentage of incidence at 35% and 39% of incidence, respectively. The mean number of nematodes for Pitt was 77 and for Harnett 78 RKN per sample. Greene (n=517) and Halifax (n=461) both had 19% positive incidence of RKN. However, the mean number of RKN per sample for Green was 11, which was lower than the mean number of 38 RKN found per sample for Halifax.

Most soil samples submitted reported a previous crop of soybean (n=15,515) and sweetpotato (n= 15,047) (Table 2.1). Soybean and sweetpotato soil samples were submitted from 21 and 23 counties, respectively. Only 20% of samples with soybean reported as a previous crop reported incidence of RKN as opposed to the 50% reported in samples with sweetpotato as the previous crop. The average number of RKN per sample was also lower on soybean at 17 juveniles/500cc soil when compared to sweetpotato at 144 juveniles/500 cm^3 soil. Out of all previous crops included, sweetpotato also had the highest maximum number of nematodes found from a soil sample. In terms of projected risk for growing sweetpotato as the next crop, only

4.2% of soybean samples required action while 17% of sweetpotato samples required action (Table 2.2).

The lowest number of soil samples received were from sorghum (n=335) and turf (n=361). In addition, less than six counties submitted soil samples for those previous crops. All other prior hosts were reported from 10 to 14 counties. Among crops with lower sample sizes, 58.5% of soil samples with sorghum reported as the previous crop had the highest occurrence of RKN. However, the projected risk for sweetpotato from sorghum samples was 12.8%, which was the same for peanut and similar for cotton (Table 2.2). Percent occurrence across cotton and peanut samples were at 40% and 28% respectively (Table 2.1). Soil samples with turf had lower RKN incidence of 18% (Table 2.2). Projected risk for growing sweetpotato from samples listing turf as a previous host was the lowest reported out of all crops (Table 2.2). Across all crops, tobacco had the highest percentage occurrence of RKN at 58% as well as the highest projected risk for sweetpotato production at 31.5% (Tables 2.2 and 2.3). Further analysis of action required by county for sweetpotato was 19% for Harnett and Cumberland county. Johnston, Sampson, Nash, and Wilson had 15 to 18% of samples requiring action.

For the estimated variance of average RKN counts amongst samples, the random effect of year had the lowest variance at 0.09. The highest variance was seen by processing date at 1.95, which is 22 times higher than the effect of year. The variance of processing date as a random effect was higher at 0.85 when compared with the effect of last crop at 0.67, but was not significantly different.

Host range of *Meloidogyne enterolobii*

Nematode reproduction was observed for all crops and demonstrates the wide host range of *M. enterolobii* over a total of four experiments (Tables 2.3, 2.4, 2.5, and 2.6). The tomato 'Rutgers' was used as a positive control plant in all four experiments and produced mean values of 38,819, 242, 502, and 2,092 eggs per gram of root among the four tests. The ER value of tomato was significantly higher in experiment 1 when compared with the other experiments. The ER value of watermelon 'Fascination' was similar to tomato in experiment 1 (Table 2.3). In experiment 2, the ER value of tomato was statistically similar to watermelon, pepper, tobacco, hemp, and pigweed (Table 2.4). In experiment 3, the ER value of tomato was statistically similar cabbage, cotton, the peanut 'Sullivan', both soybean varieties, tobacco, and winter wheat (Table 2.5). In experiment 4, only the peanut 'Tifguard', soybean '7310RY' and sudangrass were significantly different from tomato. Outside of the control, the average ER value was the highest on cabbage 'Stonehead' in experiment 2 (Table 2.4), on pepper 'Red bull' in experiment 3 (Table 2.5), and on watermelon 'Fascination' in experiment 4 (Table 2.6). The lowest average ER value was found in sudangrass 'Piper' in both experiment 2 (Table 2.4) and experiment 3 (Table 2.5). The sweet corn cultivar 'Early sunglow' had a significantly lower ER value in both experiment 2 and 3 (Table 2.4 and 2.5) when compared to tomato. The winter wheat 'Kaskia' was not significantly different from peanut 'Sullivan'. However, while both cultivars had significantly lower ER values in experiment 1 (Table 2.3), it was not significantly different from some higher ER value cultivars. In experiments 3 and 4, the ER values of cotton and soybean 'P5018RX' were not statistically different from each other and from tomato (Table 2.5 and 2.6). Of the weeds, the ER value was significantly higher for palmer amaranth when compared to nutsedge. However, while palmer amaranth had a higher ER value in experiment 1 (Table 2.3), it

was not significantly different from nutsedge in experiment 3 (Table 2.4). Also, the ER value of signal grass was low in experiment 2 (Table 2.4), but was higher in experiment 3 (Table 2.5) and not significantly different than other higher ER value cultivars such as cabbage and tobacco.

DISCUSSION

Guava root-knot nematode has significantly impacted the sweetpotato industry in North Carolina, as indicated by a quarantine being placed to limit movement of contaminated sweetpotato seeds and plants (Ye 2018). *M. enterolobii* has the potential to be very damaging due to its wide host range and ability to infect plants resistant to other RKN species (Castagnone-Sereno 2012; Rutter et al. 2018). Knowledge of host status for plant species found in North Carolina with local *M. enterolobii* isolates is beneficial for growers when choosing crops for rotations. This is the first study to report hemp, wheat, sudangrass, and the weeds yellow nutsedge, palmer amaranth, and broadleaf signalgrass as hosts for a North Carolina *M. enterolobii* population. Palmer amaranth and yellow nutsedge have been previously described as hosts for *M. arenaria* and *M. incognita* (Rich et al. 2009).

Suitable hosts for *M. enterolobii* from the GH trial included cabbage, pepper, tobacco, and watermelon which consistently had significantly higher or had similar ER values to the tomato positive control. Brito et al. (2007) reported the cabbage 'Esculenta' to be a good host. However, the cabbage variety 'Premium' was considered a poor host for the "P8" *M. enterolobii* population from Cuba (Rodriguez et al. 2003). Previous host tests have reported cucumber, pepper, tobacco, and watermelon to be good hosts for *M. enterolobii* from Cuba, Brazil, and the US (Rodriguez et al. 2003; Bitencourt and Silva 2010; Wilcken et al. 2013; J. A. Brito et al. 2007), which was in line with the results in our experiments. Differences between host responses

could be attributed to cultivar as well as the *M. enterolobii* populations, highlighting the importance of more extensive host tests and further population studies for *M. enterolobii* with diverse isolates. As *M. enterolobii* is able to reproduce on popular weeds such as palmer amaranth and yellow nutsedge, it is recommended to keep fields weed-free even during fallow periods.

While *M. enterolobii* was able to reproduce on sudangrass and corn, the ER values were significantly lower than tomato for both experiments and could be considered poor hosts. This was similar to a previous study in which low levels of egg reproduction was found on corn for *M. enterolobii* (Rosa et al. 2012). Previous host tests have found peanut (Rodriguez et al. 2003) and wheat (de Brida et al. 2018) to be a poor hosts for *M. enterolobii*. While the peanut 'Tiguard' was significantly different from tomato in both experiments, the peanut 'Sullivan' was not significantly different from tomato in one batch experiment. In addition, wheat was only significantly different from tomato in one experiment. In the batch experiments where the ER values of wheat and the peanut 'Sullivan' was higher, the average fresh root weight was lower when compared to the root weight averages in the other batch experiments. The use of sudangrass in rotation with sweetpotato could be a viable option for growers, but more studies should be done to evaluate its effect in the field. Djian-Caporalino (2019) found that sudangrass 'Piper' to be a poor host to *M. incognita* and lowered *M. incognita* J2 populations in the field and greenhouse experiments as a biofumigant. However, incorporating cover crops may be difficult for farmers as they provide no economic value when compared to other poor hosts such as corn that could be used in rotation. Further studies on the use of cover crops for suppressing North Carolina *M. enterolobii* populations would be beneficial for organic growers without access to nematicides.

Analysis of predictive soil samples submitted to the NCDA for fields rotated with sweetpotato revealed greatest percent RKN occurrence and density in North Carolina. It is recommended to take predictive soil samples before harvesting the existing crop and before the planting of the next crop (NCDA 2014). The nematode lab at the NCDA has a turnaround time of 5 weeks from sample submission to final report (NCDA 2014). In our study, a majority of predictive samples were processed in the early spring months of March and April. Peak occurrence of RKN was found from soil samples processed in the fall months and it slowly declined in winter, with population densities being lowest during the summer months. The effect of the processing month, accounted for the most variance of RKN density counts. A recent study done in the Pacific Northwest collecting data from five nematode diagnostic laboratories found a majority samples being processed during the fall months of September and October (Zasada et al. 2019). However, the number of samples processed per year ranged from 4,000-8,000, and the study only considered diagnostic samples (Zasada et al. 2019).

Several *Meloidogyne enterolobii*-positive counties were also found to have the highest mean population density of RKN such as Johnston, Wilson, Nash, and Sampson (Ye 2018). These counties were reported to have the highest acreage of tobacco harvested from 8,420 to 12,500 acres, but sweetpotato acreage was not reported for all these counties (USDA-NASS 2018). While Johnston, Wilson, Nash, and Sampson had the highest mean population density of RKN, less than 20% of samples submitted from those counties had high enough levels to recommend taking action for the next crop of sweetpotato. Wayne and Greene were the only *Meloidogyne enterolobii*-positive counties with a low occurrence and population density for RKN, which may suggest less RKN prevalence in those counties. However, the number of samples submitted for Wayne was 9 times less than Nash, which submitted the highest number

of samples (18,000 samples) from 2014-2018. In addition, only 16 individual agents, growers, or consultants submitted samples for Wayne county while Nash had 38 submissions to the NCDA. In addition, Wayne county had more farms and a higher harvested cropland acreage than Nash (USDA-NASS 2018). Soil samples analyzed in this study were not systematically taken but submitted by growers, agents, and consultants who may be aware of nematode problems in a field or crop. A soil sampling survey of targeting 28 counties where soybean cyst nematode (SCN) has not been previously detected found 5 counties with positive occurrence of SCN (Ye 2017). A systematic soil sampling survey from diverse fields would be helpful to verify the population dynamics of RKN across all counties. Columbus county, another *Meloidogyne enterolobii*-positive county, was not included in this analysis since less than 200 sweetpotato related predictive soil samples were submitted.

In general, crop rotations, which include corn or soybean with sweetpotato, may keep RKN populations low. A variation of corn, cotton, peanut, sorghum, soybean, sweetpotato, tobacco, and wheat are used for crop rotation in North Carolina (Osmond and Austin 2019). The percentage occurrence and population density by previous crop indicated that corn, soybean, and turf, have less RKN when compared to cotton, peanut, sorghum, sweetpotato, and tobacco. In addition, the projected risk for sweetpotato as a next crop showed a smaller percentage of action required if the previous host was corn, soybean, and turf. Previous studies have found RKN to reproduce on corn, cotton, cannabis hemp, and sorghum (Bowen et al. 2008; Kirkpatrick and Sasser 1984; de Meijer 1993; Hurd and Faske 2017). While the NCDA survey data have shown a higher percentage occurrence and population density for certain crops, many factors including the variety of crop grown, species or population of RKN, sampling date, and soil type may influence the outcome of that analysis. RKN-resistant varieties are available for corn,

cotton, peanut, sweetpotato, and tobacco (Nyczepir and Thomas 2009), but growers may not be using resistant varieties. In addition, the field may have *Meloidogyne enterolobii*, which is able to reproduce on crops that carry common resistance genes to major species of *Meloidogyne* (Castagnone-Sereno 2012).

In North Carolina, reproduction of *Meloidogyne enterolobii* has been reported on cotton and soybean (Ye et al. 2013). *Meloidogyne enterolobii* was observed in the greenhouse trial, to reproduce less on corn and sudangrass. Members of the Poaceae family, which includes sweet corn and sudangrass may be suitable rotational crops to manage *Meloidogyne enterolobii* if it has been detected in the field. With that said, more extensive variety evaluations of some hosts should be performed for diverse North Carolina populations of *Meloidogyne enterolobii*. Ye et al. (2013) did not report root galls on peanut, we found limited *Meloidogyne enterolobii* reproduction on the peanut cultivar 'Tifguard'. In addition, significant differences of RKN reproduction were found amongst corn and sorghum varieties (Davis and Timper 2000; Hurd and Faske 2017).

While current soil sample analysis provides a glimpse into the trends and distribution of RKN, further systematic soil samplings and analysis should be conducted to quantify population dynamics for *Meloidogyne enterolobii*. Although *Meloidogyne enterolobii* has a lower rate of reproduction on poor hosts, its potential long-term survival rate in the soil may adversely affect yields of the following crop. Previous field studies have shown different stages of RKN to survive overwinter in fallow fields (Starr and Jeger 1985). Further greenhouse studies on the pathogenicity and virulence of *Meloidogyne enterolobii* on different hosts and cultivars, as well as field studies to determine the efficacy of utilizing poor *Meloidogyne enterolobii* hosts for crop rotation with sweetpotato would be beneficial to growers.

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Table 2.1 Percentage occurrence, maximum population density, and mean population density of *Meloidogyne* spp. found from predictive soil samples[a] by different previous crops from North Carolina from 2014 to 2018.

Previous Crops[b]	Root-knot Nematode				Total Samples	Planted Acreage[d]
	Occurrence (%)	Maximum	Mean	Standard Error		
Corn	35	2,080	38.9	4.94	832	910,000
Cotton	40	3,840	78.3	6.28	1,491	430,000
Peanut	28	12,040	88.2	5.46	4,766	102,000
Sorghum	52	1,520	58.5	8.87	335[c]	18,000
Soybean	20	5,520	17.4	0.7	15,515	1,650,000
Sweetpotato	50	22,080	144.2	3.91	15,047	82,000
Tobacco	58	14,040	314.8	10	7,203	152,750
Turf	18	1,160	10.3	4	316[c]	NA

[a] Soil sample size was 500 cm^3 of soil

[b] Only previous crops with more than 200 total number of samples were considered

[c] Samples reported from 10 or less counties

[d] Harvested acreage reported from 2018 State Agriculture Overview for North (USDA NASS 2018)

Table 2.2 Projected risk for sweetpotato as a next crop according to previous crop host from predictive North Carolina soil samples[a] submitted from 2015-2018.

Previous Crops[b]	**Recommended action[c]**			**Total Samples**
	Action required (%)	**Marginal level (%)**	**No action (%)**	
Corn	9.4	25.8	64.8	837
Cotton	16.7	23.7	59.6	1,491
Peanut	12.8	15.3	71.9	4,766
Sorghum	12.8	39.4	47.8	335
Soybean	4.2	15.8	80	15,593
Sweetpotato	17	29.7	53.3	7,995
Tobacco	31.5	26.4	42.1	7,203
Turf	1.6	16.8	81.6	316

[a] Soil sample size was 500 cm^3 of soil

[b] Only previous hosts with more than 200 total number of samples were considered

[c] Recommended actions were assigned to soil samples with a rating of A = no action, B = marginal action, C = action required. Samples requiring more action had higher risk of nematode damage if growers did not take action. Ratings were determined as previously described (Ye 2015).

Table 2.3 Eggs per gram of fresh root (ER) of *Meloidogyne enterolobii* recovered from crops and weeds found in North Carolina in greenhouse experiment 1.

Common name and cultivar	**Scientific name**	**Family**	**Eggs[z]**	**FRW (g)[y]**	**Eggs/root (g)[x]**
Tomato 'Rutgers'	*Lycopersicon esculentum*	Solanaceae	100,780	10	38,819 a[w]
Cucumber 'Arabian'	*Cucumis sativus L.*	Cucurbitaceae	4,000	8.9	642 b
Hemp 'Felina'	*Cannabis sativum* L.	Cannabaceae	3,765	6.8	307 bc
Palmer amaranth	*Amaranthus palmeri* L.	Amaranthaceae	689	10.9	65.3 cd
Peanut 'Sullivan'	*Arachis hypogaea* L.	Fabaceae	48.9	16.1	4.6 e
Peanut 'Tifguard'	*Arachis hypogaea* L.	Fabaceae	114	19.3	6.1 e
Watermelon 'Fascination'	*Citrullus lanatus* Thunb.	Cucurbitaceae	62,727	5.8	11,411 a
Winter wheat 'Kaskia'	*Triticum aestivum* L.	Poaceae	73.7	68.6	1.5 e
Yellow nutsedge	*Cyperus esculentus* L.	Cyperaceae	732	85.6	10.7 de

[z] Mean values (n=5) of total eggs

[y] Mean values (n=5) of fresh root weight (FRW)

[x] Mean values of (n=5) of eggs per gram of root (ER)

[w] Values of ER were fit to a negative binomial distribution using a generalized linear model, values followed by the same letter do not differ significantly ($P < 0.0001$) by estimated marginal means

Table 2.4 Eggs per gram of fresh root (ER) of *Meloidogyne enterolobii* on crops and weeds found in North Carolina, experiment 2.

Common name and cultivar	**Scientific name**	**Family**	**Eggs**[z]	**FRW (g)**[y]	**Eggs/root (g)**[x]
Tomato 'Rutgers'	*Lycopersicon esculentum*	Solanaceae	5,700	22.1	242 bc[w]
Broadleaf signalgrass	*Brachiaria platyphylla* (L.) Beauv.	Poaceae	11.0	26.2	0.6 d
Cabbage 'Stonehead'	*Brassica oleracea* var. *capitata* L.	Brassicaceae	20,110	9.3	3,330 a
Corn 'Early sunglow'	*Zea mays* L.	Poaceae	77	32.5	2.8 d
Hemp 'Felina'	*Cannabis sativum* L.	Cannabaceae	364	20.3	59.1 c
Palmer amaranth	*Amaranthus palmeri* L.	Amaranthaceae	750	18.9	39.2 c
Pepper 'Red bull'	*Capsicum annuum* L.	Solanaceae	1,395	22.6	64.3 c
Tobacco 'K326'	*Nicotiana tabacum* L.	Solanaceae	5,540	7.0	599 ab
Watermelon 'Charleston gray'	*Citrullus lanatus* Thunb.	Cucurbitaceae	1,560	90.6	67 c
Yellow nutsedge	*Cyperus esculentus* L.	Cyperaceae	144	129	1.4 d

[z] Mean values (n=5) of total eggs
[y] Mean values (n=5) of fresh root weight (FRW)
[x] Mean values of (n=5) of eggs per gram of root (ER)
[w] Values of ER were fit to a negative binomial distribution using a generalized linear model, values followed by the same letter do not differ significantly ($P < 0.0001$) by estimated marginal means

Table 2.5 Eggs per gram of fresh root (ER) of of *Meloidogyne enterolobii* on crops and weeds found in North Carolina, experiment 3.

Common name and cultivar	Scientific name	Family	Eggs[z]	FRW (g)[y]	Eggs/root (g)[x]
Tomato 'Rutgers'	*Lycopersicon esculentum*	Solanaceae	2,080	5.1	503 b [w]
Broadleaf signalgrass	*Brachiaria platyphylla* (L.) Beauv.	Poaceae	65.8	3.7	16.11 cde
Cabbage 'Stonehead'	*Brassica oleracea* var. capitata L.	Brassicaceae	332	2.5	155.5 b
Corn 'Early sunglow'	*Zea mays* L.	Poaceae	53.2	6.1	7.4 de
Cotton 'Deltapine 1646'	*Gossypium hirsutum* L.	Malvaceae	1,076	5.0	185 b
Peanut 'Sullivan'	*Arachis hypogaea* L.	Fabaceae	112	2.8	36.9 abcd
Pepper 'Red bull'	*Capsicum annuum* L.	Solanaceae	57,394	6.4	12,810 a
Soybean '7310RY'	*Glycine max* (L.) Merrill	Fabaceae	682	7.7	86.7 bc
Soybean 'P5018RX'	*Glycine max* (L.) Merrill	Fabaceae	645	9.4	75.0 abc
Sudangrass 'Piper'	*Sorghum bicolor* (L.) Moench	Poaceae	6.7	8.1	1.9 e
Tobacco 'K326'	*Nicotiana tabacum* L.	Solanaceae	766	3.7	225.8 b
Winter wheat 'Kaskia'	*Triticum aestivum* L.	Poaceae	117	15.1	39.4 bcd

[z] Mean values (n=5) of total eggs

[y] Mean values (n=5) of fresh root weight (FRW)

[x] Mean values of (n=5) of eggs per gram of root (ER)

[w] Values of ER were fit to a negative binomial distribution using a generalized linear model, values followed by the same letter do not differ significantly ($P < 0.0001$) by estimated marginal means

Table 2.6 Eggs per gram of fresh root (ER) of *Meloidogyne enterolobii* on crops and weeds found in North Carolina, experiment 4.

Common name and cultivar	Scientific name	Family	Eggs[z]	FRW (g)[y]	Eggs/root (g)[x]
Tomato 'Rutgers'	*Lycopersicon esculentum*	Solanaceae	20,472	8.8	2,092 b
Cucumber 'Arabian'	*Cucumis sativus L.*	Cucurbitaceae	5,679	3.9	1,396 bc
Cotton 'Deltapine 1646'	*Gossypium hirsutum* L.	Malvaceae	3,378	6	574 bc
Peanut 'Tifguard'	*Arachis hypogaea* L.	Fabaceae	343	10.0	30.7 d
Soybean '7310RY'	*Glycine max* (L.) Merrill	Fabaceae	2,903	9.3	281 c
Soybean 'P5018RX'	*Glycine max* (L.) Merrill	Fabaceae	4,308	6.2	706 bc
Sudangrass 'Piper'	*Sorghum bicolor* (L.) Moench	Poaceae	32.0	20.0	1.9 e
Watermelon 'Charleston gray'	*Citrullus lanatus* Thunb.	Cucurbitaceae	1,823	1.3	1,580 b
Watermelon 'Fascination'	*Citrullus lanatus* Thunb.	Cucurbitaceae	18,366	1.2	18,717 a

[z] Mean values (n=5) of total eggs

[y] Mean values (n=5) of fresh root weight (FRW)

[x] Mean values of (n=5) of eggs per gram of root (ER)

[w] Values of ER were fit to a negative binomial distribution using a generalized linear model, values followed by the same letter do not differ significantly ($P < 0.0001$) by estimated marginal means

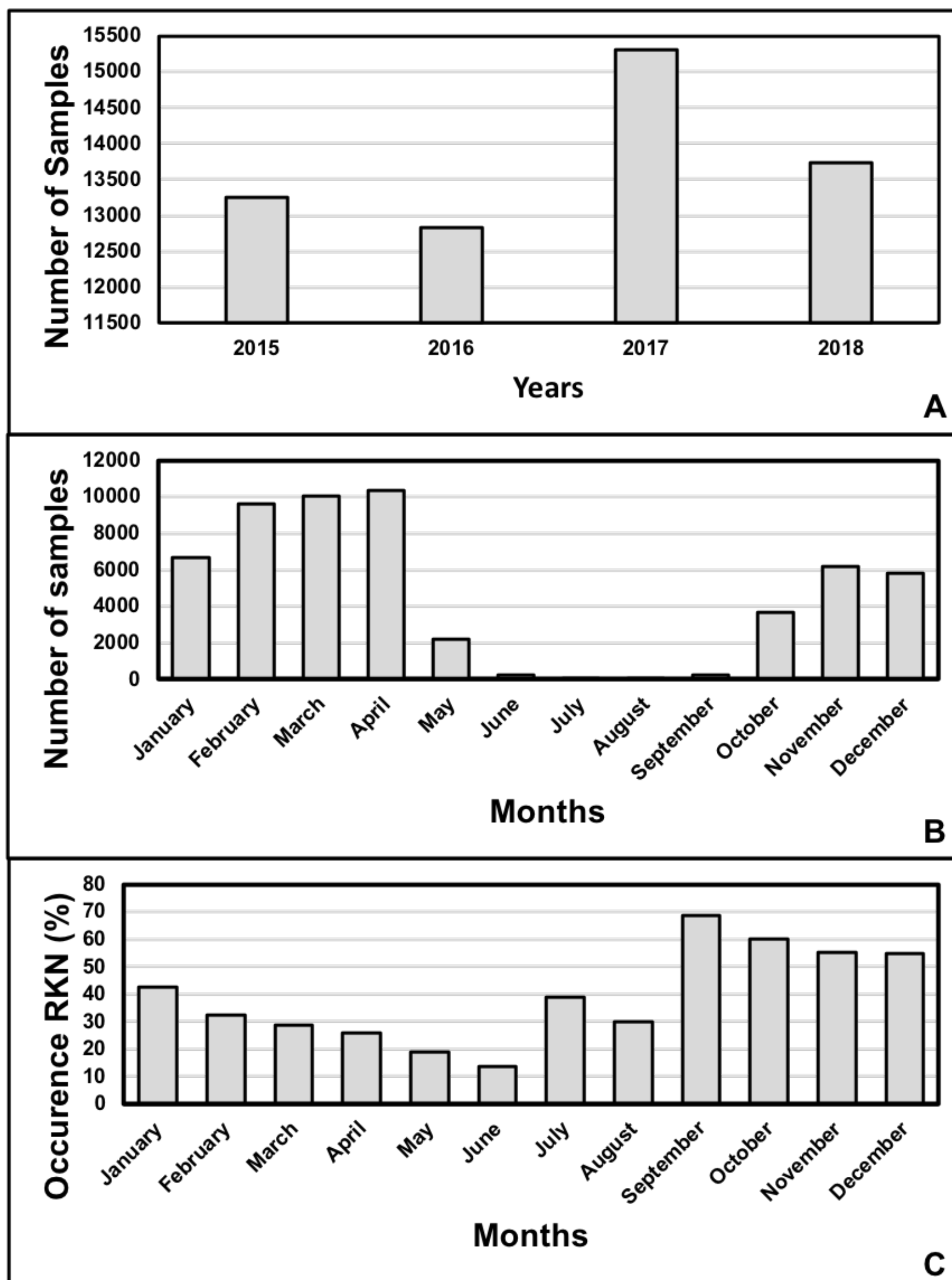

Figure 2.1 Histograms of the **A.** number of samples submitted by year, **B.** number of samples processed by month, and **C**. the percentage occurrence of RKN found from samples by month for predictive nematode soil samples from 2014 to 2018 in North Carolina (n = 55,127).

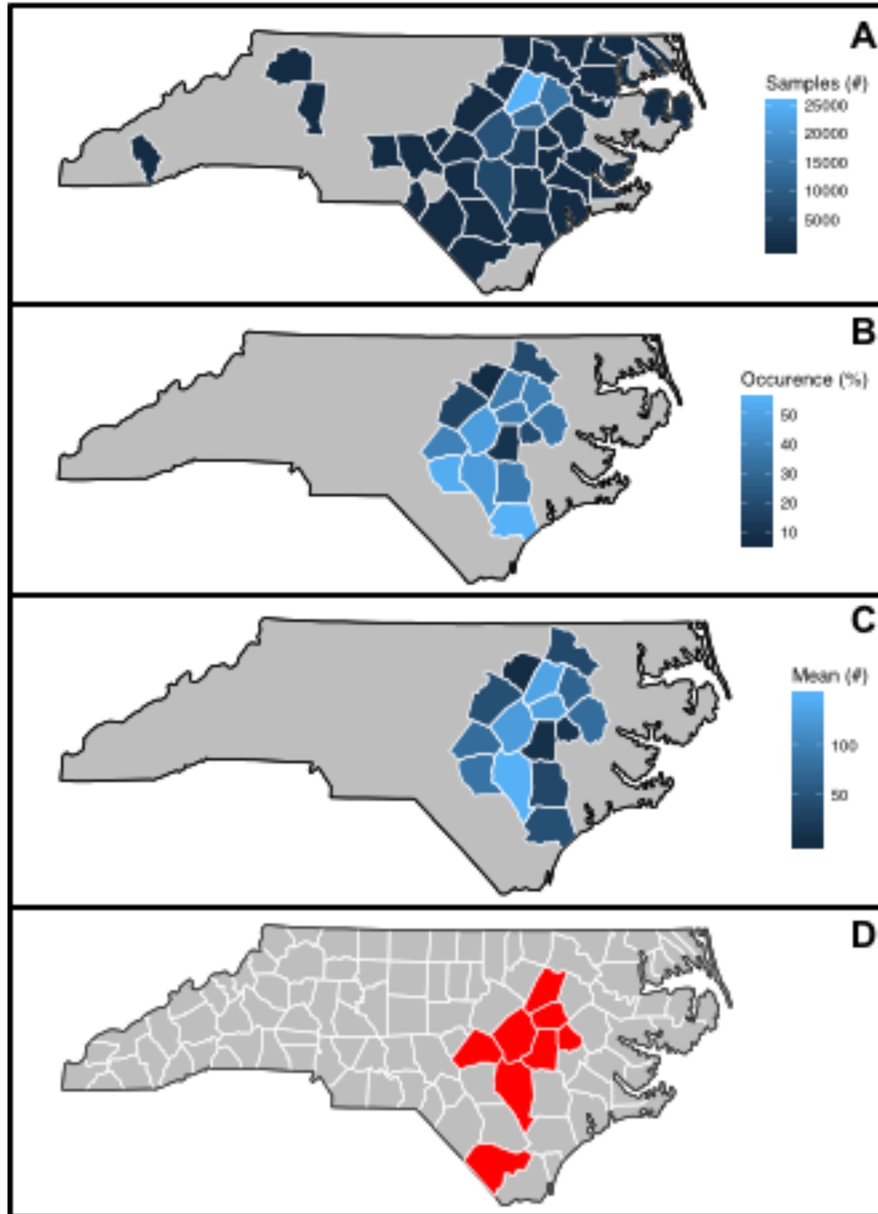

Figure 2.2 Map of **A.** the total number of predicted samples submitted, **B.** the percentage occurrence (%) of *Meloidogyne* spp. across counties submitting more than 100 samples containing 500 cm^3 of soil, **C.** the average count of *Meloidogyne* spp. per 500 cm^3 of soil across counties submitting more than 100 samples, and **D.** counties with reported incidence of *M. enterolobii*. Samples had were submitted to the NCDA from 2015 to 2018 in North Carolina.

CHAPTER 3

Sensitivity of *Meloidogyne incognita*, *Fusarium oxysporum* f.sp. *niveum*, and *Stagonosporopsis citrulli* to succinate dehydrogenase inhibitors used for control of watermelon diseases

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ABSTRACT

Watermelon, an important crop grown in several regions of the United States (US), is affected by diseases such as Fusarium wilt, gummy stem blight, and root-knot nematode. Newly developed succinate dehydrogenase inhibitors (SDHIs) with potential fungicide and nematicide activity provide the opportunity to control multiple diseases with one compound in watermelon; nonetheless, the sensitivity of multiple watermelon pathogens to these active ingredients has not been investigated. In this study, we aimed to determine the sensitivity of *Meloidogyne incognita* race 4 (MI4), *Fusarium oxysporum* f.sp. *niveum* (FON), and *Stagonosporopsis citrulli* (SCIT), to existing and new SDHIs: benzovindiflupyr, fluopyram, an experimental, and pydiflumetofen. Assays targeting two life stages were conducted for FON and MI4. Cross-sensitivity for SDHIs were also determined for the fungal pathogens. All SDHIs had fungicidal activity against 19 SCIT isolates, but isolates were most sensitive to pydiflumetofen (median EC_{50} = 0.41 µg/ml). A total of 50 FON isolates were tested and most were sensitive to the experimental in both the mycelial growth (median EC_{50} = 4.04 µg/ml) and conidial germination (median EC_{50} = 0.2

μg/ml) assay. However, significant positive correlations between the sensitivity of the experimental to benzovindiflupyr (r =0.7, P< 0.0001) and experimental to pydiflumetofen (r =0.77, P< 0.0001,) indicate a potential for cross-resistance between these SDHIs. Fluopyram was not efficacious against most FON isolates. MI4 was most sensitive to the experimental in both the egg hatch (mean EC_{50} = 0.0019 μg/ml) and J2 motility assays (mean EC_{50} = 1.16 μg/ml), but was not sensitive to pydiflumetofen in either assay. Overall, results suggest that the experimental may be used for managing root-knot nematode, whereas it should be used judiciously for Fusarium wilt of watermelon and gummy stem blight due to the existence of insensitive isolates to the fungicide.

INTRODUCTION

The United States (US) is one of the top ten producers and consumers of watermelon with South Carolina and North Carolina being the 6th and 7th largest producers (FAOSTAT 2017). In the Southeastern region of the US, watermelon production can be limited by pathogens such as root-knot nematodes (RKNs), Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON), and gummy stem blight (GSB) caused by *Stagonosporopsis citrulli* (SCIT) (syn. *Didymella bryoniae*), *S. cucurbitacearum*, and *S. caricae* (Brewer et al. 2015). Management of these pathogens requires a combination of chemical and cultural methods; however, cultural practices are limited due to the distribution and wide host range of FON and RKN in the soil and the lack of commercial resistant watermelon varieties against all three pathogens (Thies et al., 2016; Costa et al., 2018; Gusmini et al., 2005). Field and environmental conditions may facilitate multiple diseases that occur concurrently, particularly in the case of RKN interacting with other soil-borne pathogens, thus research into other multi-pathogen control options would be beneficial for watermelon disease management (Manzanilla-López and Starr 2009).

Fusarium wilt and GSB are regarded as two of the most important diseases in watermelon production in the US (Kousik et al., 2016). *F. oxysporum* f.sp *niveum* is a soil-borne fungal pathogen and few effective fungicides exist for Fusarium wilt. Currently, only prothioconazole (Proline 480 SC; Bayer CropScience, Research Triangle Park, NC), part of the demethylation inhibiting (DMI) fungicide group [Fungicide Resistance Action Committee (FRAC) code 3], is labeled to manage Fusarium wilt on watermelon (Miller et al.in press). Gummy stem blight is a foliar disease that affects all above-ground parts of the watermelon (Keinath 2013). *Stagonosporopsis citrulli* (syn. *Didymella bryoniae*) has been reported as the most widespread GSB pathogen in the US (Keinath and DuBose 2017). Although multiple fungicides of various

chemical classes are available for managing GSB, *Stagonosporopsis* spp. are considered high-risk pathogens for developing resistance due to their short life cycle and frequent fungicide applications over a growing season (Thomas et al. 2012, Keinath 2015). Southern root-knot nematode (*Meloidogyne incognita*) is considered the major RKN species impacting crop production in the Southeastern US and most watermelon cultivars are highly susceptible to most RKN species (Thies and Levi 2007b). A global survey funded by USAID found that 18-33% crop loss of cucurbits in the tropics is due to nematodes (Sasser 1979) and regional studies have shown watermelon crop losses to be as high as 50% (Lamberti 1979).

New fungicide chemistries in the succinate dehydrogenase inhibitor (SDHI) class have been recently registered or are pending registration for disease control in watermelon, presenting new opportunities for effective management of gummy stem blight, Fusarium wilt, and root-knot nematodes. These new SDHI active ingredients (a.i) include benzovindiflupyr, fluopyram, pydiflumetofen, and an experimental compound (under development and pending registration). While previous SDHIs were labeled for multiple fungal pathogens, fluopyram, labeled as a fungicide, was also found to have nematicidal properties against root-knot nematode, reniform nematode, and soybean cyst nematode (Faske and Hurd 2015; Heiken 2017). These SDHI fungicides (FRAC group 7) are categorized as medium to high risk for resistance development due to their single mode of action of inhibiting cellular respiration by targeting the complex II of the mitochondria (Sierotzki et al., 2011). For example, boscalid-resistant field isolates of *Stagonosporopsis* spp. were found in Georgia and South Carolina a few years after coming into market (Thomas, Langston, Sanders, et al. 2012; Keinath 2012). Currently, no known populations of FON isolates from North Carolina with resistance to SDHIs have been found, but SDHI-resistant isolates have been documented with other fungal pathogens (Avenot and

Michailides 2010; Stammler et al. 2015; Miller et al.in press). Monitoring the sensitivity of SCIT and FON populations to these new SDHIs is important to avoid resistance development (Thomas et al. 2012). In addition, cross-resistance and a lack of cross-resistance have been reported among SDHIs across different pathogens (Avenot and Michailides 2010). A lack of cross-resistance has been documented for fluopyram and boscalid-resistant isolates for *Stagonosporpsis* spp. (Avenot et al. 2012). The potential of cross-resistance between the newer SDHIs should be explored to ensure proper fungicide rotations and disease management.

Research determining if these new SDHIs are effective against multiple watermelon pathogens would be highly beneficial in understanding SDHI effects on pathogen populations and life stages. Recent sensitivity studies for SCIT have only been conducted for fluopyram and only one study has been done for the sensitivity of FON isolates to pydiflumetofen (Thomas et al. 2012; Keinath 2012; Li et al. 2019; Miller et al.in press). Beyond these studies, little is known about the pattern of SCIT and FON population sensitivity to the newer SDHI chemistries and the potential cross-resistance between the newer SDHI fungicides. In addition, screening the efficacy of these SDHIs for managing root-knot nematodes is important to understand its role as a potential non-fumigant chemical control option in watermelon and other crops. To address these needs we aimed to: i) determine the effects of SDHIs on mycelial growth and microconidial germination of FON collected from California, Florida, Indiana, Maryland, North Carolina, South Carolina, and Texas, ii) determine the effects of SDHIs on mycelial growth of *Stagonosporopsis* spp. isolates recovered from infected watermelon in North and South Carolina, and iii) establish the sensitivity of *Meloidogyne incognita* race 4 (MI4) to SDHIs based on motility of the second-stage juvenile (J2) infective stage and egg hatching assays.

MATERIALS AND METHODS

Fusarium oxysporum f. sp. *niveum* (FON) and *Stagonosporopsis citrulli* (SCIT) isolate collection and storage

For this study, 50 total isolates of FON were collected from watermelon grown in California (n=7), Florida (n=7), Indiana (n=5), Maryland (n=7), North Carolina (n=10), South Carolina (n=10), and Texas (n=4). For SCIT, 19 isolates were collected from butternut squash, acorn squash, and watermelon from the Southeast region (Table 3.1). Symptomatic plants were identified in the field and the infected tissue was surface sterilized in NaOCl (10%) for 30 seconds. The tissue was rinsed in sterile distilled water (SDW), cut into small pieces, and placed on a selective Nash and Snyder media (Thies and Levi 2007) for FON isolations and on quarter-strength potato dextrose agar (QPDA) for *Stagonosporopsis* isolations (Stevenson et al. 2004). After isolating from plant material, all isolates were single-spore transferred to water agar and re-plated on QPDA. After 7 days, isolates were examined microscopically and identified based on morphological features on QPDA (Keinath 2013a, Leslie and Summerell 2008). For long term storage, a plug of agar with a single spore of FON or SCIT was grown on filter paper on QPDA. The isolate filters were stored in -20ºC in a sterilized sealed coin envelope. *Fusarium* isolates were verified to be FON using FON1 and FON2 specific PCR primers (Lin et al. 2010). *Stagonosporpsis* isolates were identified by SCIT using species-specific PCR primers Db01, Db05, and Db06 (Brewer et al., 2015).

Sensitivity of fungal mycelial growth to succinate dehydrogenase inhibitors (SDHIs)

The four SDHIs tested included an experimental compound (38.5% a.i.), Velum Prime (41.5% a.i of fluopyram, Bayer CropScience), Miravis (18.3% a.i. of pydiflumetofen, Syngenta

Crop Protection), and Aprovia (10.27% a.i. of benzovindiflupyr, Syngenta Crop Protection). The commercial formulations of all SDHIs were dissolved in sterile water and used to make stock solutions of 1, 10, 100, 1,000, 10,000, and 100,000 mg formulation/ml. QPDA media was amended with the SDHIs or water to a final concentration of 0, 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml. After being cooled to 60ºC, the amended media was poured in 60 x 15mm petri dishes.

Fifty isolates of FON and nineteen isolates of *S. citrulli* were grown on QPDA at 25ºC in sealed clear containers on a 12h light/dark cycle for 5 days. A mycelial plug (4mm in diameter) was taken from the edge of each colony using a cork borer and placed on the SDHI-amended and control plates. Two replicates were used for each SDHI-concentration treatment and the control. When the mycelial growth of the non-amended control plates reached the edges, two perpendicular colony diameter measurements were taken for each treatment plate associated with that isolate. Two independent trials were run for each isolate. Percent relative mycelial growth was calculated from the mean colony diameter on fungicide amended media divided by the mean colony diameter on the control plate and multiplied by 100.

Sensitivity of FON fungal germination to SDHIs

Fifty isolates of FON were grown on QPDA at 25ºC in sealed clear containers on a 12h light/dark cycle for 7 days. Sterile distilled water (SDW) was added to each plate and a sterile glass spreader was used to gently harvest the spores. The spore suspension was filtered through two layers of sterile cheesecloth and collected in a 15ml sterile falcon tube. Using a hemocytometer, the concentration was determined and adjusted to 1 x 10^4 spores/ml with SDW. Water agar media was amended with the four SDHI formulations as described above or water-control to a final concentration0, 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml after being cooled to

60ºC in 60 x 15mm petri dishes. A volume of 70 µl of spore suspension of each FON isolate was spread on the SDHI-amended and nonamended (control) water agar plates. Two plates were used for each fungicide concentration and isolate and two independent trials were run for each isolate. After incubation for 24h, the microconidia and macroconidia were stained with lactophenol cotton blue. One hundred microconidia were randomly counted on each plate as germinated or non-germinated in one area. Conidia were considered germinated if the germ tube length was more than half of the conidia. The relative germination rate of each isolate was calculated by dividing the percent germination of each concentration-dose by the average percent germination of the control for that particular run, multiplied by 100.

Sensitivity of *Meloidogyne incognita* race 4 Juveniles and Eggs to SDHIs

Meloidogyne incognita race 4 (MI4) isolated from North Carolina was maintained on roots of 'Rutgers' tomatoes in greenhouse culture for at least 60 days before harvesting eggs. Eggs of MI4 were extracted from tomato roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973) and collected on a 25 micron-opening sieve. The second-stage juveniles (J2) of MI4 were hatched from the eggs on a Baermann pan at 27C (Heiken 2017). A J2 motility assay (Heiken 2017) was used to determine the effect of SDHIs on the cohorts of hatched MI4 J2s. The J2 were treated for two hours with water solutions amended with the four SDHI formulations described above or water-control to a final concentration of 0, 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml in a 15ml glass cull culture tube. After the treatment solution of two hours, the nematodes were triple rinsed with water and collected using a 10 micron-opening sieve. Approximately 50-75 nematodes were placed in an individual well of a 48-well tissue culture plate. At 24 hours after rinsing, five wells were systematically counted for each concentration using grid marks

marked on the culture plate and two trials were run for each SDHI. The number immotile or straight nematodes out of 50 worms was recorded after 1, 8, and 24 hours. The relative survival rate of J2s was calculated by dividing the percent survival of each concentration-dose by the average percent survival of the control well after 24 hours of exposure and multiplied by 100.

A hatch assay using the modified Baermann pan technique (Vang et al. 2016) was used to determine the effect of SDHIs on the egg stage in the MI4 lifecycle. An estimated 5,000 eggs of MI4 were pipetted in a hatching pan with hatching water solutions amended with the SDHI formulations above or water to a final concentration of 0, 0.001, 0.01, 0.1, 1, 10, and 100 μg/ml.. After 5 days in a 27C incubator, the total number of hatched J2s were counted. Three hatching pans were used for each concentration and two hatching trials were run for each SDHI. The relative hatch rate of eggs was calculated by dividing the number of J2s of each concentration-dose by the average number of J2s from controls for each run and multiplied by 100.

Data Analysis

The concentration of the experimental, fluopyram, pydiflumetofen, and benzovindiflupyr that effectively inhibited the pathogen by 50% (EC_{50}) for all assays was estimated by linearly regressing relative inhibition values (i. e. 100 – relative growth) on log_{10}-transformed fungicide concentrations using PROC REG in SAS (version 9.6, SAS Institute, Cary, NC). Afterwards, a nonparametric Wilcoxon signed-rank test was performed on the $log_{10}(EC_{50})$ values for paired runs with PROC UNIVARIATE to assess reproducibility and if experimental repeats could be combined. Frequency distributions of the estimated EC_{50} values were examined using PROC UNIVARIATE with the Shapiro-Wilk test. For the FON assay (n=50), the $log_{10}(EC_{50})$ values of the germination and mycelial were compared by state (CA, FL, IN, MD, NC, SC, and TX) using

a nonparametric Wilcoxon rank-sum test with PROC NPAR1WAY followed by a post-hoc Bonferonni adjustment of the rank data with PROC GLM in SAS. The Spearman's rank-order correlation (PROC CORR) was used for SCIT and both FON assays to determine the relationship between all SDHIs and to evaluate the potential for cross-sensitivity between SDHIs.

RESULTS

The sensitivity of 50 *F. oxysporum* f. sp. *niveum* (FON), 19 *S. citrulli* (SCIT) isolates from the US to existing and new SDHIs was determined by using mycelial growth and spore germination assays. The identity of all FON isolates was confirmed using species specific primers FON1 and FON2 that produced a 174-bp DNA fragment. The identity of 19 SCIT was confirmed using primers Db06, which produced a 285-bp fragment for SCIT and Db01, which produced a 360-bp fragment for SCIT isolates (Table 3.1).

Effect of SDHIs on mycelial growth and spore germination of *F. oxysporum* f. sp. *niveum* and *S. citrulli*

The log_{10}-transformed EC_{50} values of individual isolates between experimental repeats for SCIT mycelial assay as well as the FON mycelial and germination assay were combined after determining they were not significantly different from the Wilcoxon signed-rank test. Normality was assessed from the frequency distributions of the log_{10}-transformed EC_{50} values and the assumptions were met for all fungicides. All SDHIs reduced mycelial growth of the 19 isolates of SCIT included in this study. Pydiflumetofen had the lowest median EC_{50} value (0.41 µg/ml) with the smallest range of EC_{50} values from 0.03 to 1.82 µg/ml (Table 3.2). The experimental

compound had the highest median EC_{50} value (1.35 μg/ml) as well as the largest range of EC_{50} values from 0.05 to 12.3 μg/ml. Fluopyram and benzovindiflupyr had estimated median EC_{50} values of 1.10 and 1.21 μg/ml, respectively. Spearman's rank-order correlation was used since the data was non-parametric. The correlation of mean EC_{50} values between fluopyram and experimental and fluopyram and benzovindiflupyr were not significant (Table 3.3). However, a weak positive correlation of mean EC_{50} values was found between fluopyram and pydiflumetofen ($r = 0.54$, $p < 0.01$). The strongest correlation of mean EC_{50} values in the SCIT mycelial assay were between the experimental to benzovindiflupyr ($r = 0.88$, $P < 0.0001$) and the experimental to pydiflumetofen ($r=0.83$, $P < 0.0001$).

In the FON mycelial assay, 98% and 90% of isolates had estimated EC_{50} values above the highest tested concentration (above 100 μg/ml) to fluopyram and benzovindiflupyr, respectively. Thus, fluopyram and benzovindiflupyr were considered less effective for this life stage and not included in the FON mycelial analysis. Frequency distributions of the $\log_{10}$-transformed EC_{50} values were normal for the experimental ($[Pr < W] = 0.13$) and pydiflumetofen ($[Pr < W] = 0.8$). For the experimental, the EC_{50} values ranged from 0.58 to 24.29 μg/ml and the median EC_{50} value was 4.04 μg/ml (Figure 3.1A; Table 4). EC_{50} values for isolates exposed to pydiflumetofen had a larger range from 0.89 to 41.02 μg/ml and a higher median EC_{50} value of 6.29 μg/ml (Figure 3.1B; Table 3.4). Mean EC50 values of isolates from North and South Carolina were significantly less sensitive to pydiflumetofen, while isolates from Maryland and Florida were the most sensitive ($P < 0.001$) (Table 3.5). For the experimental, North Carolina isolates were significantly the least sensitive while isolates from Maryland, Florida, Indiana, and California were most sensitive ($P < 0.001$).

In the FON conidial germination assay, 66% of isolates exposed to fluopyram had mean EC_{50} values above the tested concentration range and were considered insensitive to that SDHI. Several isolates insensitive to fluopyram were also insensitive to the experimental compound and pydiflumetofen (Figure 3.12). However, one isolate was very sensitive to fluopyram, the experimental fungicide, and pydiflumetofen and had a mean EC_{50} value below 0.001 μg/ml. Isolates exposed to benzovindiflupyr had EC_{50} values that ranged from 0.012 to 57.29 μg/ml, but values did not meet the assumption for normality ($[Pr < W] = 0.003$). When the isolates outside of the tested concentration for fluopyram, experimental, and pydiflumetofen were excluded from analysis, the distribution of $log_{10}(EC_{50})$ values followed a normal distribution. Isolates exposed to the experimental had the lowest EC_{50} median (0.2 μg/ml), followed by pydiflumetofen (1.03 μg/ml), and benzovindiflupyr (4.53 μg/ml) (Table 3.4). While the median EC_{50} level for the germination assay was lower than mycelial, the range of germination EC_{50} values was much wider than for the mycelial assay. Mean EC_{50} values of isolates between states were not statistically different for the experimental ($P > 0.05$). For benzovindiflupyr and pydiflumetofen, mean EC_{50} values of isolates from South Carolina were lower ($P < 0.05$). Mean EC_{50} values of California isolates were higher for pydiflumetofen as four out of the 7 tested isolates had mean values above 5 μg/ml (data not shown). Spearman's rank-order correlation indicated a positive association between the sensitivity of FON to all SDHIs (Table 3.6). The strongest correlations were in the germination assay between the experimental to benzovindiflupyr ($r = 0.7$, $P < 0.0001$) and NMG to pydiflumetofen ($r = 0.77$, $P < 0.0001$).

Effects of SDHIs on *Meloidogyne incognita* motility and egg hatch assays

The log_{10}-transformed EC_{50} values generated from experimental repeats were combined for the MI4 motility and egg hatch assays after determining they were not significantly different from one another. The MI4 EC_{50} value for pydiflumetofen for both assays and benzovindiflupyr for the motility assay could not be calculated (value above 100 µg/ml). However, MI4 eggs in the hatch assay were sensitive to benzovindiflupyr with an EC_{50} value of 1.05 µg/ml (Table 3.7). Both MI4 life stages assessed were most sensitive to the experimental, which had an EC_{50} value of 0.0018 µg/ml for the egg hatch assay and a value of 1.16 µg/ml for the J2 motility assay. The EC_{50} value generated from the fluopyram motility assay was 10.06 µg/ml, which was higher than the EC_{50} value for the hatch assay 0.088 µg/ml. Motility recovery was observed after the 2h hour exposure for three SDHIs. At the 100 µg/ml concentration, an increase in motility of live nematodes was observed for benzovindiflupyr (79%), fluopyram (54%), and the experimental compound (13%) (data not shown).

DISCUSSION

The range of mean EC_{50} values generated for SCIT isolates from the Southeast region were wider than previously reported EC_{50} ranges based on mycelial growth assays for the baseline sensitivities of the SDHI boscalid and penthiopyrad for isolates collected from watermelon leaves in Georgia (Thomas et al. 2012). The range of fluopyram (0.43 – 4.46 µg/ml) from our study was also much wider and not within reported range of fluopyram (0.02 to 0.189µg/ml) mean EC_{50} values for 26 SCIT isolates from watermelon fields in Georgia, North Carolina, and South Carolina in 2009 (Avenot et al. 2012).. During the time of those previous studies, it was not known that GSB was comprised of three different species, but phylogenetic

and population studies suggest that most isolates collected from the southeastern US prior to 2013 were SCIT (Brewer et al. 2015). Isolates in this study were collected from 1998 to 2013 and were collected from watermelon, butternut squash, and acorn squash leaves from more than one state, which may explain the wider EC_{50} value ranges for all SDHIs. At least half of the isolates were from 2013 and may have had more exposure to SDHIs, which may explain the wider EC_{50} ranges of fluopyram in this study. Differences in formulation could also explain wider of the EC_{50} values from this study. Commercial formulation, which may have included fungicide additives, was used in this study as opposed to the technical formulation, which only contained the active ingredient. The EC_{50} values for the experimental reported in our study constitute the baseline sensitivity for this SDHI since SCIT isolates have not been previously exposed to this unregistered active ingredient (Table 3.2). SDHI fungicides target the ubiquinone-binding pocket in the enzyme succinate dehydrogenase reductase, which is comprised of the subunits SDHB, SDHC, and SDHD. Mutations in any of these three subunits may alter the shape of the pocket and confer resistance to SDHIs (Sierotzki and Scalliet 2013). The ranges of EC_{50} values in this study were more similar to the wider distributions reported recently for isolates of *Botrytis cinerea* with key mutations in the SDHB subunit for fluopyram (Amiri et al. 2014). The relatively wider ranges of EC_{50} values in this study may indicate some variations in the SDH enzyme within the SCIT population in regards to collection date, location, as well as plant host.

Cross-sensitivity is commonly observed among fungicides belonging to the same FRAC group due to their common mode of action. Since all four SDHIs tested in this study are in different SDHI chemical groups, examining the potential for cross-sensitivity For SCIT isolates, this study also revealed a positive correlation of sensitivity to pydiflumetofen to each of the

SDHIs: benzovindiflupyr, the experimental compound, and fluopyram. A strong positive association between the sensitivity of benzovindiflupyr and the experimental compound was also observed. Cross-sensitivity between pydiflumetofen and fluopyram is surprising as previous studies have previously reported a lack of cross-resistance has for fluopyram to other SDHIs for SCIT, *Alternaria alternata*, *B. cinerea*, and *Corynespora cassiicola* (Avenot et al. 2012; Avenot and Michailides 2010; Veloukas and Karaoglanidis 2012; Ishii et al. 2011). However, in more recent studies, cross-resistance of fluopyram to penthiopyrad has been reported in *B. cinerea* and *Venturia inaequalis* (Amiri et al. 2014; Villani et al. 2016; Ayer et al. 2018). Despite observed cross-sensitivity between SDHIs, it cannot be concluded that cross-resistance will occur between the fungicides. For example, cross-resistance was not observed in *V. inaequalis* between DMI fungicides myclobutanil and difenoconazole even though cross-sensitivity was observed between them in baseline populations (Villani et al. 2015). With everything considered, monitoring shifts in sensitivity levels of SCIT populations to newer SDHIs is extremely important as cross-resistance has been observed over time between SDHIs. For SCIT, a mutation in SDHB has been identified for an isolate resistant to fluopyram (Li et al. 2019). Further studies characterizing amino acid substitutions and cross-resistance patterns between SDHIs for SCIT populations would be highly beneficial for fungicide spray programs in gummy stem blight management.

The sensitivity to SDHIs was determined for both microconidial germination and mycelial growth for FON isolates. Pydiflumetofen and the experimental had most activity against FON isolates in both assays while benzovindiflupyr was only effective in the conidial assay. The range of mycelial EC_{50} values for FON isolates exposed to pydiflumetofen in our study (0.89 – 41.02 µg/ml) was higher than previously reported values (0.34 – 3.68 µg/ml) (Miller et al.in press), likely due to differences in isolates used. Miller et al. (in print) used

isolates from North Carolina with 8 isolates from other locations while this study included 4-10 isolates from 7 states. In addition, fluopyram was not effective against FON, which was contrary to sensitivity studies for other *Fusarium* species. The range of mycelial EC_{50} values for *F. virgluforme* to fluopyram ranged from 1.53 to 9.28 µg/ml and the range of macroconidial germination was 0.81 to 5.57 µg/ml (Wang et al. 2016). In addition, the mycelial EC_{50} range to fluopyram from three other *Fusarium* species associated with soybean sudden death syndrome ranged from 0.06 to 4.24 µg/ml (Sang et al. 2018). Differences seen between *Fusarium* spp. for mycelial EC_{50} values may be a result of differences in the SDH enzyme.

In the mycelial assay, a positive correlation was found between the EC_{50} values of the experimental and pydiflumetofen. A significant and positive correlation between sensitivities to the experimental and that of the two other SDHIs effective in the microconidial germination study were also observed. Further research determining the potential mechanisms of SDHI fungicide resistance in FON isolates would be beneficial in understanding variations of sensitivity and potential cross-resistance among isolates. If cross-resistance between the experimental compound and pydiflumetofen is confirmed in future studies, the use of the experimental in areas with widespread pydiflumetofen resistance may not be desirable.

This is the first report for a microconidial germination assay for SDHI sensitivity in FON. The range of EC_{50} values from the microconidial germination assay was wider than for mycelial growth as one isolate was found to be very sensitive. In addition, several isolates were found to be insensitive (> 100 µg/ml) to one or more SDHIs in the microconidial germination assay despite mycelial growth inhibition. Despite these outliers, it seemed SDHIs were more effective inhibiting spore germination for FON. Median EC_{50} values for the experimental and pydiflumetofen were 20 and 6 times greater for mycelial growth than for microconidial

germination, respectively. It has been previously shown that the level of inhibition of SDHIs towards different growth stages such as conidial germination and mycelial growth have been inconsistent (Amiri et al. 2010; Veloukas and Karaoglanidis 2012; Vega and Dewdney 2015; Villani et al. 2016). Sensitivity levels of the SDHI fungicide boscalid was higher for mycelial growth when compared to conidial germination for *A. alternata* (Vega and Dewdney 2015) and *Monilinia fructicola* (Amiri et al. 2010). However, conidial germination of fluopyram was more sensitive for *Venturia inaequalis* (Villani et al. 2016; Ayer et al. 2018) and *B. cinerea* (Veloukas and Karaoglanidis 2012). SDHI compounds exhibit different binding efficacy and that efficacy may vary depending on variations of the ubiquinone pocket within populations and across pathosystems (Sierotzki and Scalliet 2013). Higher germination sensitivity to fluopyram when compared to mycelial sensitivity was also reported in *Fusarium virgiliforme* even though fluopyram was largely ineffective against FON (Wang et al. 2016). The relatively higher levels of efficacy demonstrated by pydiflumetofen and the experimental compound to conidial germination against FON isolates suggest that these compounds should provide better control as a protective agent to prevent conidial germination.

The effects of fluopyram and benzovindiflupyr have been previously determined for *M. incognita* J2 motility (Faske and Hurd 2015), but sensitivities were yet to be determined for the experimental compound and pydiflumetofen. To our knowledge, this is the first report investigating the sensitivity of multiple SDHIs on two life stages of MI4. Our results revealed that the experimental compound was the most effective SDHI in targeting the egg and J2 stages of the MI4 lifecycle. Pydiflumetofen and benzovindiflupyr were not effective in the J2 motility test, but benzovindiflupyr was effective in the MI4 hatch assay. Most nematicides for RKN focus

on the J2 stage that is present in treated soil and less on other stages of its life cycle (Nordmeyer 1992).

Previous studies with *Caenorhabditis elegans* have found fluopyram and benodanil, another SDHI fungicide, to have nematicidal properties (Burns et al. 2017; Heiken 2017). We found the experimental compound (EC_{50} 1.16 µg/ml) to be more effective than fluopyram (EC_{50} 10.06 µg/ml) in our motility assay with MI4 after an exposure of 2h and a recovery of 24h. Motility assays of *M. incognita* with fluopyram reported a higher EC_{50} value (5.18 µg/ml) after 2h exposure and a lower value (1.18 µg/ml) after 24h exposure (Faske and Hurd 2015). Differences in EC_{50} values may be attributed to the different SDHI formulations as are likely due to the *M. incognita* populations used in either study, highlighting the importance of incorporating pathogen diversity when possible to account for phenotypic differences among populations. For example, Oka and Saroya (2019) showed baseline sensitivity of 2 separate populations of *M. incognita* from Israel to be significantly different from each other after exposure to fluensulfone in a motility test. Furthermore, sensitivity to SDHIs across different species of nematode varies as was found with *M. javanica* being more sensitive to fluopyram (Oka and Saroya 2019) and *R. reniformis* being less sensitive to fluopyram (Faske and Hurd 2015). While resistance from long-term exposure in *M. incognita* towards nonfumigant nematicides has been reported (Meher et al. 2009),, studies with *C. elegans* showed resistance to SDHIs was unlikely (Burns et al. 2015). Future research to determine susceptibility levels of SDHIs across more plant parasitic nematodes, species, and populations within a species is needed.

Our research also saw reversible J2 paralysis in MI4 after 2-hour exposure to fluopyram, the experimental compound, and benzovindiflupyr. Faske and Hurd (2015) had similar results of J2 revival of MI and *R. reniformis* exposed to fluopyram. By contrast, the recovery assay from

Oka and Saroya (2019) showed irreversible immobilization of *M. incognita* and *M. javanica* after 48 hours of exposure to fluopyram at 4 mg/L, which suggests that effects of SDHIs to *Meloidogyne* spp. at a longer exposure at a higher concentration are nematicidal. Studies have been performed to investigate the mode of action of SDHIs in nematodes. Using a *Caenorhabditis elegans* mutant with knockout of the *sdh* gene, Heiken (2017) reported increased sensitivity in this nematode model organism to fluopyram. However, Faske and Hurd (2015) also speculated SDHIs' mode of action to interfere with the chemoreception of the nematode after finding low rates of infection in tomato roots from nematodes exposed to sublethal doses of fluopyram. So far, it seems that SDHIs work as a contact nematicide on J2 soil as seen with the in vitro assays. Currently, applications for fluopyram, labeled as Velum Prime to control nematodes, include soil drenching and chemigation.

For multi-purpose control of fungal pathogens and nematodes, SDHIs would be a viable option in watermelon disease management. Grafting has been previously proposed as a management option for fields with both FON and RKN (Keinath and Agudelo 2018) but the current expense of grafted plants remains an obstacle for industry-wide adoption. In this study, the experimental compound was the most effective SDHI in terms of multi-purpose control for FON and MI4. However, pydiflumetofen was more effective against SCIT and several isolates were found to be less sensitive to the experimental compound. Among FON isolates, a positive correlation for cross-sensitivity was found between the experimental compound to pydiflumetofen– the experimental compound may soon become registered for Fusarium wilt control in watermelon (Miller et al.in press). The results of this study indicate that the experimental compound would not be a desirable option in areas with reported FON isolates that are insensitive to pydiflumetofen. Since SDHIs work as a contact nematicide (Faske and Hurd

2015), the experimental compound would be ideal as a drench treatment for watermelon in fields with FON and MI4. A foliar spray of SDHIs to control GSB would not be effective against RKNs since it is a contact nematicide; however, SDHIs have a diverse range of chemistry and, depending on the host and pathogen, have protectant, translaminar or systemic activity (McKay et al. 2011). Further research is needed to understand SDHI mobility in watermelon to determine if a foliar application could manage FON. In addition, due to our findings of less sensitive isolates to some SDHIs and potential cross-insensitivity to other, it will be important to develop programs that discourage appearance of fungicide resistance in pathogen populations.

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Table 3.1 Geographic origin, host of origin, and number of *Stagonosporopsis citrulli* (SCIT) and *Fusarium oxysporum* f. sp. *niveum* (FON) isolates used in this study.

Pathogen	**State**	**Host**	**No. of isolates**
SCIT	Southeast region	Butternut squash	3
		Acorn squash	4
		Watermelon	12
	Total		19
FON	California	Watermelon	7
	Florida		7
	Indiana		5
	Maryland		7
	North Carolina		10
	South Carolina		10
	Texas		4
	Total		50

Table 3.2 Effective concentration per fungicide (µg/ml) that reduced *Stagonosporopsis citrulli* (19 isolates) relative mycelial growth by 50% (EC_{50})

Fungicide	Mean	Median	Range	Normality (Pr < W) [a]
Benzovindiflupyr	1.18	1.21	0.14 – 5.99	0.404
Fluopyram	1.23	1.10	0.43 – 4.46	0.604
Experimental	1.19	1.35	0.05 – 12.3	0.317
Pydiflumetofen	0.41	0.41	0.03 – 1.82	0.074

[a]Measure of normality using Shapiro-Wilk test

Table 3.3 Correlation[a] of mean EC_{50} values for SDHI fungicides from a mycelial assay for 19 isolates of *Stagonosporopsis citrulli*.

Fungicide	**Fungicide**	**r**	***P***
Pydiflumetofen	Benzovindiflupyr	0.7	0.003
Pydiflumetofen	Fluopyram	0.54	0.01
Pydiflumetofen	Experimental	0.83	< 0.0001
Fluopyram	Benzovindiflupyr	0.15	0.44
Fluopyram	Experimental	0.38	0.1
Experimental	Benzovindiflupyr	0.88	< 0.0001

[a] Spearman's rank-order correlation

Table 3.4 Effective concentration per fungicide (µg/ml) that reduced *Fusarium oxysporum* f. sp. *niveum* (50 isolates) relative mycelial growth and microconidial germination by 50% (EC_{50})

Assay	**Fungicide**	**Mean**	**Median**	**Range**
Mycelial	Experimental	4.12	4.04	0.58 – 24.29
	Pydiflumetofen	7.14	6.29	0.89 – 41.02
Germination	Benzovindiflupyr	4.33	4.53	0.012 – 57.29
	Fluopyram	<100	<100	<0.001 – >100
	Experimental	0.42	0.2	<0.001 – >100
	Pydiflumetofen	<100	1.03	<0.001 – >100

Table 3.5 Mean EC_{50} values and standard errors per fungicide (µg/ml) from mycelial assay by state for 50 isolates of *Fusarium oxysporum* f. sp. *niveum*.

	Fungicide	
State	**Experimental**	**Pydiflumetofen**
California	3.50 ± 1.11 a[z]	4.90 ± 1.25 ab
Florida	2.61 ± 1.32 a	2.79 ± 1.31 a
Indiana	3.26 ± 1.4 a	4.74 ± 1.23 ab
Maryland	2.73 ± 1.24 a	2.75 ± 1.22 a
North Carolina	8.59 ± 1.22 b	17.16 ± 1.21 c
South Carolina	5.34 ± 1.15 ab	13.77 ± 1.20 c
Texas	5.74 ± 1.22 ab	10.05 ± 1.40 bc

[a] Mean values and standard error followed by the same letter are not significantly different according to the Wilcoxon rank sum and Bonferroni multiple comparisons statistical test ($P < 0.05$).

Table 3.6 Correlation[a] of mean EC_{50} values for SDHI fungicides from mycelial and germination assay for 50 isolates of *Fusarium oxysporum* f. sp. *niveum*

Assay	**Fungicide**	**Fungicide**	**r**	***P***
Mycelial	Pydiflumetofen	Experimental	0.67	< 0.0001
Germination	Pydiflumetofen	Experimental	0.77	< 0.0001
	Pydiflumetofen	Benzovindiflupyr	0.58	< 0.0001
	Experimental	Benzovindiflupyr	0.7	< 0.0001

[a] Spearman's rank-order correlation

Table 3.7 Mean EC_{50} values per fungicide (µg/ml) of egg hatch and J2 motility assay for *Meloidoyne incognita* race 4

Assay	Fungicide	Mean
Egg[a]	Benzovindiflupyr	1.05
	Fluopyram	0.088
	Experimental	0.0019
	Pydiflumetofen	>100
J2[a]	Benzovindiflupyr	>100
	Fluopyram	10.06
	Experimental	1.16
	Pydiflumetofen	>100

[a] Mean EC_{50} calculated after 5 days of exposure

[a] Mean EC_{50} calculated after a 24-hour recovery period following a 2 hour exposure

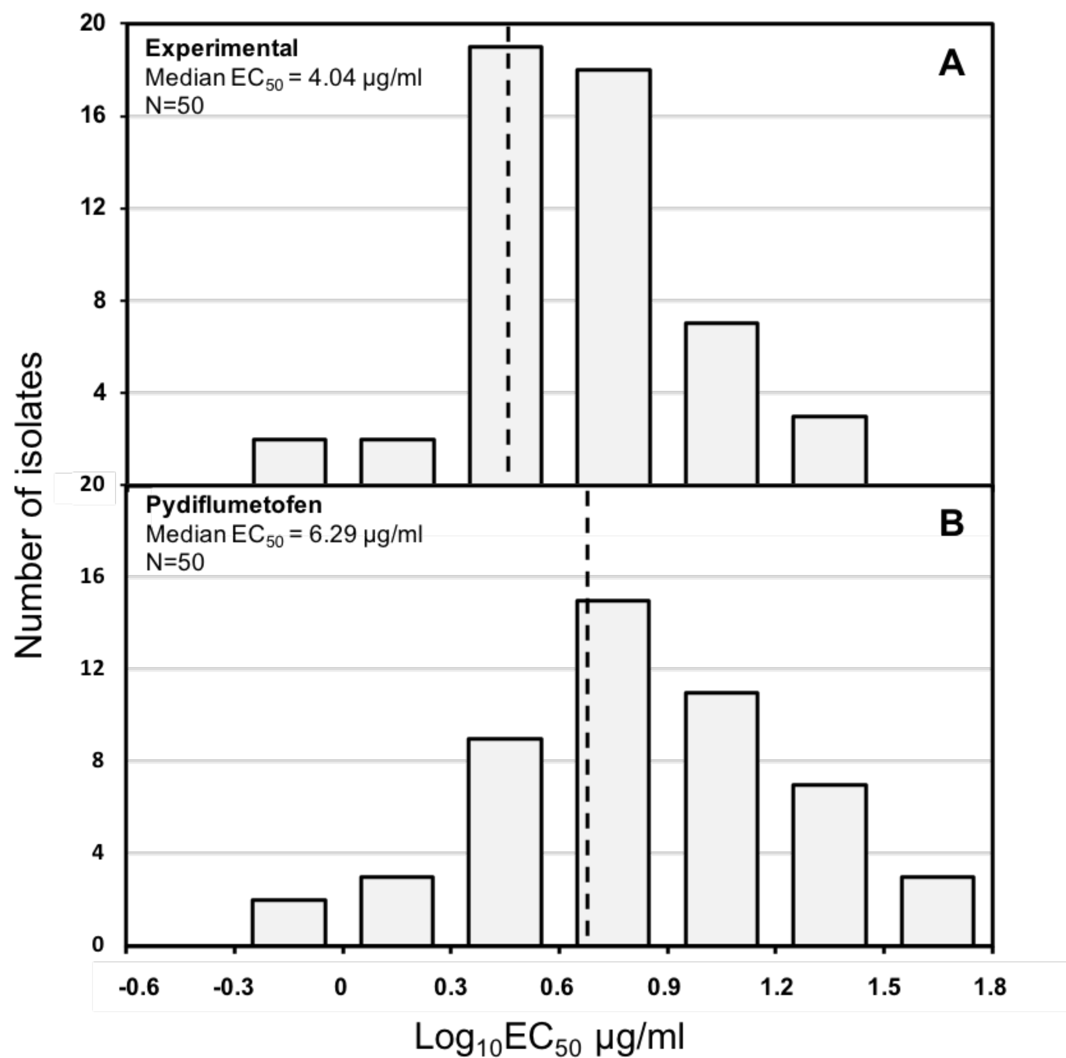

Figure 3.1 Frequency distribution of the effective concentration at which mycelial growth was inhibited by 50% (EC_{50} values) in isolates of *Fusarium oxysporum* f. sp. *niveum* for **A**. the experimental and **B.** pydiflumetofen.

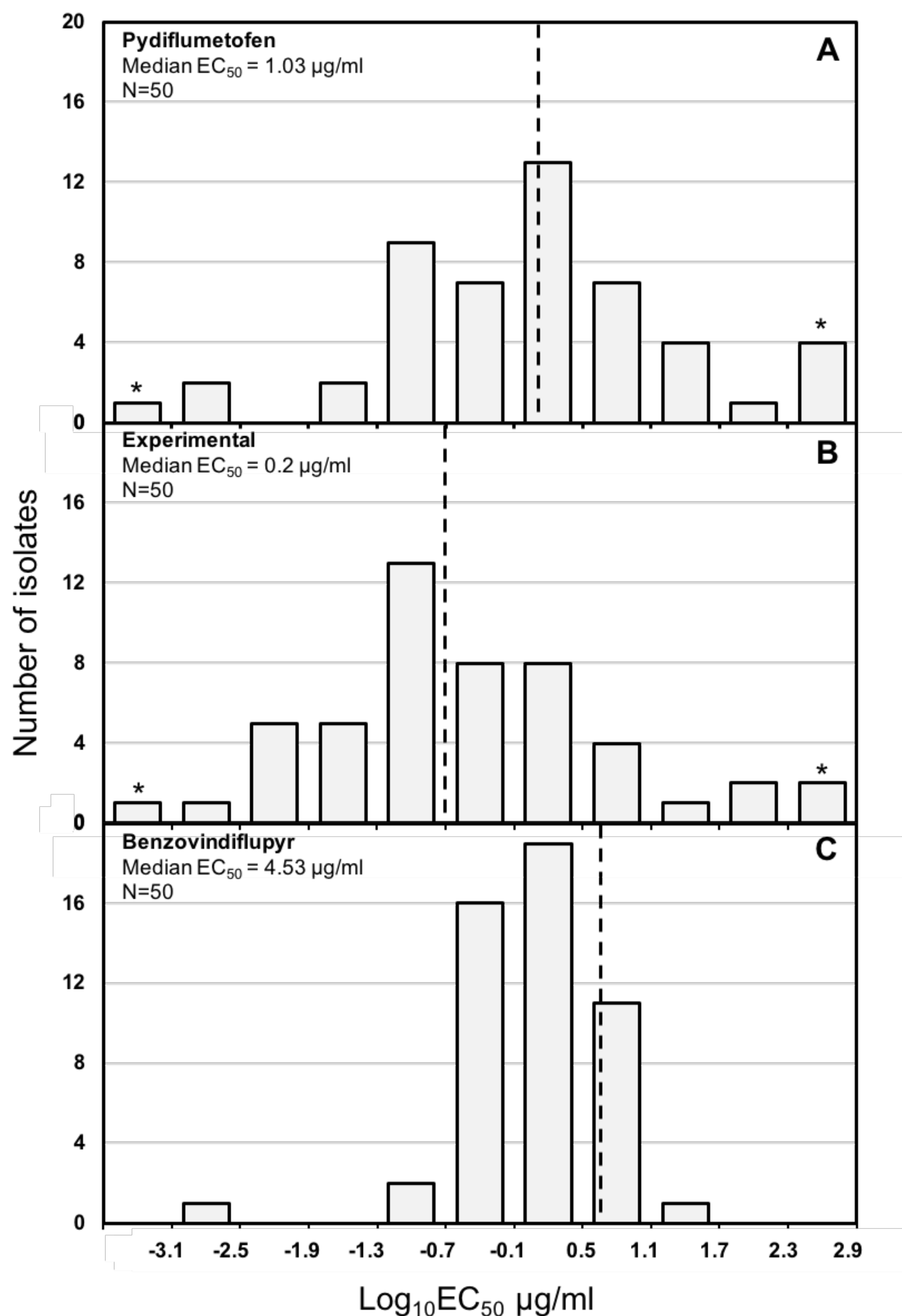

Figure 3.2 Frequency distribution of the effective concentration at which microconidia germination was inhibited by 50% (EC_{50} values) in isolates of *Fusarium oxysporum* f. sp. *niveum* for **A**. pydiflumetofen, **B.** experimental, and **C.** benzovindiflupyr. The asterisk (*) indicates isolates with EC_{50} values less than or higher than the concentrations tested in this study.