Southern Root-knot Nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) and Fungal Susceptibility of Turmeric (*Curcuma longa* L.) Accessions

by

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Abstract

Turmeric (*Curcuma longa* L.), a spice crop native to southeast Asia, is undergoing evaluation as a niche crop for Alabama and other southeastern U.S. production areas. Thirteen accessions of *Curcuma* spp. (including eight accessions of C. *longa*, common turmeric) are cultivated as a part of the Auburn University Medicinal Plant Collection on the campus of Auburn University. Rhizomes harvested from the turmeric plots during the 2015-2016 production season showed symptoms of rhizome rot, and mature plants exhibited stunting, chlorosis, and root galling, characteristic of *Meloidogyne incognita* (Kofoid and White) Chitwood infestation. Fungi were isolated from diseased rhizomes and post-harvest soil samples were taken to identify plant-parasitic nematodes present. Fungal genera isolated included *Fusarium* (60.9%), *Rhizoctonia* (21.9%), *Trichoderma* (9.7%), *Macrophomina* (2.5%), *Diplodia* (2.5%), and *Nigrospora* (2.5%). Fungal sequences were amplified by PCR, and forty-one isolate identities were confirmed. Koch's postulates were completed for *Rhizoctonia solani* on *C. longa*. *Meloidogyne incognita* was identified throughout the turmeric plots and documented as the first case of *M. incognita* on *C. longa* in the United States.

Greenhouse trials were conducted to assess the growth of three *C. longa* accessions with and without the presence of *M. incognita*. At 60 days after inoculation, *M. incognita*-inoculated turmeric accessions CL2 and CL7 exhibited significantly reduced average plant height, shoot fresh weight, and root fresh weight with the measurements being 31%, 50%, and 26% of those of the control, respectively. Final nematode population density on CL2, CL3, and CL7 ranged from

19-319, 1-2527, and 41-4703 eggs per gram of root, respectively. Reproductive factor (RF), defined as the final nematode population density divided by the initial inoculum density, was calculated to be 0.6, 4.1, and 2.1 for CL2, CL3, and CL7, respectively. Consequently, turmeric accessions CL3 and CL7 were susceptible to the nematode, as their RF values were greater than 1. Greenhouse and microplot trials were conducted in the summer and fall of 2016 to further assess the susceptibility of *C. longa* accessions to *M. incognita*. *Curcuma longa* 2, *C. longa* 4, and *C. longa* 7 exhibited improved growth over other accessions. All accessions tested were good hosts of *M. incognita* with reproductive factors of 3.9-10.4. Fields trials were conducted to evaluate potential nematicides for *M. incognita* management. All nematicide treatments had a *M. incognita* population density of 34-477 eggs per gram of root, and *C. longa* biomass was similar to the untreated control. Because all turmeric selections grown on the campus of Auburn University are susceptible to *M. incognita* and nematicides are not a labeled for control, *M. incognita* may prove a major pest in Alabama turmeric production. More research will be required to establish best management practices, potentially including more chemical and biological nematicides, soil amendments, and expanded variety selection.

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Chapter 1: Review of Literature

Statement of Purpose

As the spice turmeric becomes more relevant as both a fresh market and processed human health supplement, this research aims to identify accessions of turmeric that will grow well in nematode- and fungal-infested soils in Alabama.

A Brief History of Turmeric

The Vedic culture in India first recorded use of turmeric six thousand years ago in both culinary and religious practices. A paste made of the ground rhizomes was traditionally smeared on the skin of brides of India, Bangladesh, and Pakistan to enhance skin radiance and deter harmful microbes; a similar concoction was applied to birthing mothers and the umbilical cord of newborns to discourage "evil spirits" (Prasad and Aggarwal, 2011). For the past 2,000 years and even today, Buddhist monks of Southeast Asia, recognized by their saffron-colored attire, have used the spice as a dye to color their robes (Lal, 2012). The prized plant became dispersed throughout much of the known world, reaching China by 700 A.D. and Africa a century later. Marco Polo mentioned turmeric when recording his travels, noting the rhizome as a vegetable with properties of saffron (Prasad and Aggarwal, 2011). Though the plant was carried to Europe, the Americas, and the Caribbean over the next one thousand years, turmeric did not spark interest in these new societies until German researchers in the 1920s began extracting compounds from rhizomes for therapeutic evaluation (Lal, 2012). Arora et al. (1971) observed turmeric's ability to reduce inflammation in mice comparable to standard prescription treatment hydrocortisone, and Kuttan et al. (1985) found turmeric useful in inhibiting cancerous tumor

growth and reducing overall tumor growth in mice and providing symptomatic relief in humans with skin cancer (Kuttan et al., 1987). Curcumin, the most widely studied and marketed turmeric compound, is referenced in more than 5,600 citations in peer-reviewed scientific literature (Aggarwal et al., 2013), and for ample reason. Curcumin supplement intake can result in decreased risk of pre-diabetic individuals from developing diabetes mellitus (Chuengsamarn et al., 2012), induction of apoptosis of tumorous and cancerous cells (Zheng et al., 2016), and reduction of arthritis symptoms (Daily et al., 2016). These health benefits associated with turmeric are most likely associated with the ability to scavenge free radicals, reduce iron complexes to an absorbable form in the blood, and prevent peroxidation (Tilak et al., 2004).

Introduction to *Curcuma longa*

Curcuma longa is classified as a member of Zingiberaceae, a family of monocots known as the "ginger" family. In addition to the family's namesake, ginger (*Zingiber officinale*), other spices including cardamom (*Elettaria cardamomum* Maton.) and large cardamom (*Amomum sublatum*) belong to this group (Prabhakaran Nair, 2013). Ginger and turmeric rhizomes appear quite similar at first glance in regards to size and shape; however, the primary chemical constituent of the rhizomes are a notable difference between the genera. Aforementioned curcumin is identified as the most important chemical constituent of turmeric, whereas gingerols are the equivalent in ginger (Ramirez-Ahumada et al., 2006). Because both spices are affected by similar pathogens, and as information regarding ginger is more widely available than turmeric, ginger will be mentioned throughout this review.

Turmeric is the common name of plants belonging to the 133 *Curcuma* species, with the foremost species identified as *Curcuma longa* (Prasad and Aggarwal, 2011). Forty known species of the *Curcuma* genus trace origins to India with a remaining 70-110 species collected

and identified throughout the southeastern Asian continent (Velayudhan et al., 1999). The spice crop is commonly produced in tropical and sub-tropical regions with average temperatures between 20-30˚C and ample rainfall. The plant can grow in a wide range of soil types but thrives in sandy or clay loams with adequate drainage and sufficient organic matter (Jayashree et al., 2015). Mature plants stand one meter tall with large, oblong leaves with a bright green hue.

Below the soil line, roots emerge from mother rhizomes and absorb water and nutrients in the soil. A portion of the root system may become enlarged and fleshy as a means of storing water and food materials. The powdered spice known as turmeric is derived from rhizomes, or underground stems, that form below the soil line. Rhizomes can be differentiated as central, pear-shaped "mother" rhizomes or lateral-growing "daughter" rhizomes that branch off from the mother (Prabhakaran Nair, 2013). Either rhizome type, if not processed for culinary, medicinal, or cosmetic use, can be used for seed material the following season (Prasad and Aggarwal, 2011). At least 2,500 kilograms of seed material is required for planting one hectare of turmeric, and virtually all sowing is done by hand. Maximum rhizome development requires 7-9 months; because most turmeric production falls within tropical latitude, plants are usually sown in April-July and harvested in January-April. Mature rhizomes can be harvested by manual digging or a turmeric harvester pulled by a tractor, but both methods require collecting the dug rhizomes by hand (Jayashree et al., 2015). Once harvested, rhizomes undergo a 15-20 day curing process before preservation or processing for seed material (Lal, 2012). Typical yield ranges from 25-30 metric tons per hectare of fresh rhizomes or 5-6 metric tons per hectare of cured rhizomes (TNAU, 2013). Rhizomes vary in length from 2.5 to 7 centimeters with a papery, segmented skin. The interior of *C. longa* cultivars displays a red-orange color, but the flesh of rhizomes of other species are white, pink, purple, green, blue, or black (Prasad and Aggarwal, 2011).

Once cured, rhizomes may be sold as a fresh market item or processed into value-added products including dehydrated powder, turmeric oil, oleoresin (organic extract used to color foods), or curcuminoids (pharmaceutical and coloring agent) (Prabhakaran Nair, 2013).

Current Production Areas

Over one million tons of turmeric are produced each year with 80% of production concentrated in India; remaining production areas include regions of Jamaica, Sierra Leone, Nigeria, Malaysia, China, and Japan (Eapen et al., 2008). All major Indian production states, Andra Pradesh, Tamilnadu, Orissa, West Bengal, Maharashtra, Karnataka, and Kerala, saw increases in cultivated land area from 2003-2012, and all states except Maharashtra increased total turmeric yield during this time (Peshwe and Gelda, 2015).

Although central Alabama's latitude (32-33˚N) lies slightly north of India's latitude (10-30˚N), environmental conditions including adequate rainfall, sandy soil types, and mild temperatures form a conducive environment for turmeric production. A burgeoning fresh market demand currently exists in Alabama; not only are consumers seeking out and purchasing turmeric at farmers markets around the state, they are requesting varieties of turmeric high in curcumin (T. Gonzalez, personal communication, 2016).

Pests of Production: Nematodes

General nematode infestation symptoms include stunting, chlorosis of leaves, and poor shoot development (Greco and Di Vito, 2009). Plants inoculated with the root-knot nematode exhibited similar symptoms as well as marginal leaf necrosis and a significantly reduced number of shoots compared to control plants after six months (Ramana and Eapen, 1995). Specific nematodes can induce characteristic symptoms that can be helpful for identifying the pathogen. Feeding of burrowing nematodes (*Radopholus similis*) produces shallow, water soaked

depressions along roots that result in rotting of the root cortex. *Radopholus* can also infect rhizomes intracellularly which, if saved for seed, can ultimately lead to pathogen dissemination. Several species of the lesion nematode (*Pratylenchus* spp.) weaken root systems, and feeding sites are characterized by symptomatic brown lesions. The lesions eventually encircle the entire root and lead to shallow root systems with portions of dead roots. *Meloidogyne* spp. (predominantly *M. incognita,* but also *M. hapla* and *M. arenaria*) infection results in galling on roots caused by internal root feeding that induces plant cell hypertrophy (cell enlargement) and hyperplasia (increase in cell number). Additionally, the infected rhizomes will exhibit a dull yellow color (Ramana and Eapen, 1999). Cheng and Tu (1979) described *M. incognita* invasion of ginger through fibrous roots, fleshy roots, and rhizomes. The infective-stage juveniles (J2) enter fibrous roots through the point of differentiation but can move into fleshy roots along the entire length of the root. The axils of leaf sheaths in the shoot apex of a newly germinated rhizome present another point of entry to invading J2.

Koshy and Bridge (1990) noted that turmeric and ginger serve as plant hosts to fourteen and sixteen nematode species, respectively, and of these species, *M. incognita*, *R. similis*, and *P. coffeae* emerged as the three most economically important to Indian turmeric and ginger production systems. A study conducted by Mani and Prakash (1992) recorded seventeen different genera of plant-parasitic nematodes extracted from the rhizosphere of turmeric plants in fields of Andhra Pradesh, India. *Rotylenchulus reniformis*, found in 70% of fields sampled, represented the most dominant nematode species in terms of total frequency followed by *M. incognita* (60%). A survey of turmeric production fields in the Indian state of Kerala by Bai et al. (1995) revealed the presence of five nematode species including *M. incognita*, *R. reniformis*, *Radopholus similis, Helicotylenchus* sp., and *Tylenchorhynchus* sp. Of the five nematode species,

M. incognita emerged as the prominent pest from field samples, representing 67-100% of the total nematode population density from each region. In fields of *M. incognita* infestation alone, yield loss reached as high as 45.3%. Ray et al. (1995) found that the presence of *M. incognita* reduced yield in both turmeric and ginger crops by 33.61% and 26.32%, respectively.

Establishing descriptors of loss is a vital part of the study of any host-pathogen relationship. Teng (1985) described loss as "the reduction in quantity and/or quality of the economic yield of a crop". Haider et al. (1998) evaluated inoculation level and effects of combination of *M. incognita* and *R. reniformis* on young turmeric plants. The study determined the damage threshold of both nematodes at 100 infective juveniles per 500 grams of soil, and the combination of the two nematodes at 5,000 infective-stage juveniles each resulted in the highest level of growth reduction (51.48%) of young turmeric plants. Sukumaran et al. (1986) observed reductions in shoot length and weight, lamina width, number of leaves, root weight, and rhizome yield with inoculation of > 1,000 *M. incognita* J2 per plant compared to uninoculated control plants. Furthermore, an inoculum level of 100,000 *M. incognita* J2 per plant reduced rhizome weight by 76.6% in six-month-old plants compared to control plants. In addition to yield loss, crop response to *Meloidogyne* spp. can be characterized by observation of galling on root systems either in terms of the quanitity of galls as proposed by Taylor and Sasser (1978) or the size of galls and overall root appearance as suggested by Di Vito et al. (1979).

The life cycle of *M. incognita* spans thirty days on turmeric plants at temperatures of 25-35˚C. Second-stage juveniles initially invade roots only twenty-four hours after inoculation and continue entering roots for six days; the J2 molts an additional three times before reaching the mature, predominantly female reproductive stage (Mohanta and Swain, 2014a). Reproduction of *Meloidogyne* species can vary from obligatory amphimixis (sexual reproduction) to obligatory

apomixis (asexual reproduction). Amphimitic species tend to exhibit narrower host specificity. Apomixis in *Meloidogyne*, more specifically parthenogenesis, is characterized by wide host ranges, shorter life cycles, and higher rates of reproduction. Parthenogenic species including M. incognita, though less genetically diverse, are considered the most important crop pathogens (Trudgill and Blok, 2001).

Because one nine-month growing season may give rise to as many as nine generations of nematode pathogens, nematode management practices offer crucial assistance to growers with infested fields. As of 2010, *M. incognita* has been recorded in 46 out of Alabama's 67 counties (NCC, 2010). Because of the widespread distribution of this known pest, Alabama turmeric growers should sample fields before sowing to detect the presence of any nematode pests and have knowledge of potential chemical, biological, and cultural strategies for nematode management.

Current Nematode Management Strategies

Chemical nematicides and insecticides can efficiently decrease nematode population density; however, increased environmental concerns are resulting in the discontinuation of use of older chemistries. Carbofuran™ reduced *Meloidogyne* population density in turmeric production by as much as 81.6% (Ramana and Eapen, 1999), but the chemical was classified as ineligible for reregistration in the United States by the Environmental Protection Agency (EPA) in 2007 (EPA, 2007). Additionally, fumigant methyl bromide was phased out of U.S. crop production in 2005 (EPA, 2016), and Bayer CropScience agreed to voluntarily cancel the registration of popular nematicide Temik™ (aldicarb) in 2010 (EPA, 2010).The exit of older chemistries from the market creates opportunity for alternative nematicidal options that are both effective and sustainable.

Common cultural control measures including soil solarization, heat treatment of rhizomes, and resistant varieties can effectively decrease nematode population density. Soil solarization refers to the process of covering a portion of soil with sheets of clear plastic and using solar energy to "cook" the soil. The maximum temperature of the top five centimeters of soil can range between 42-60˚C; soil temperatures to a depth of 45 centimeters usually only reach between 32-37˚C. Temperatures between 30-33˚C are lethal to most pathogens, but often solarization is most effective to a depth of 30 centimeters below the soil line (Elmore et al., 1997). This technique has been found to provide multiple benefits including weed control, improved plant stand and development, and increased yield. Solarizing soil offers a sustainable replacement to fumigation in terms of both human safety and cost to the grower (Katan et al., 1976). Soil solarization for fifteen days in the summer in India was found to lower root-knot galling index and increase yield over chemical (Carbofuran™) and organic amendment treatments (Patel et al., 2008). Dipping rhizomes in hot water, either 45˚C for 60 minutes or 50-55˚C for 10 minutes, can prevent nematode dispersal via seed material (Chen et al., 1986; Ramana and Eapen, 1999). Furthermore, nematode resistance is available in select varieties of turmeric. Indian varieties Armoor, Duggirala, Guntur-1, Guntur-9, Rajampet, Sungandham, and Upplapadu were found to demonstrate resistance to the root-knot nematode and are recommended in areas of high infestation (Ramana and Eapen, 1999). Mohanta et al. (2015) saw significantly less galling on root-knot nematode-inoculated rhizomes of cultivars 'Durigala', 'PTS-31', 'Ansitapani', 'PTS-21', and 'PTS-47', leading to the classification of these cultivars as resistant to the root-knot nematode.

Biological control tactics such as introducing antagonistic microorganisms and integration of organic amendments can be implemented as nematode management practices.

Eapen et al. (2008) investigated various fungal bioagents including *Aspergillus* sp., *Fusarium oxysporum,* and *Paecilomyces lilacinus* as a means of nematode control. Though all fungal bioagents increased plant height compared to the control, only *F. oxysporum* increased rhizome yield significantly (40.9% increase over control) in field trials. Furthermore, organic amendments have been evaluated as potential controls for nematode-infested soils. Application level of dried poultry manure in greenhouse studies inversely affected *M. incognita* nematode galling and egg production; however, high rates of poultry manure (>22 tonnes/ha) induced phytotoxicity (Udo and Ugwoke, 2010).

Mohanta and Swain (2014b) found that three combinations of integrated pest management including 1) crustacean exoskeleton amendment (0.75 tonne/ha) plus soil solarization, 2) carbofuran (0.75 kg a.i./ha) plus *Bacillus subtilis* in aqueous solution (5 L/ha), and 3) sesame oil cake (0.75 tonne/ha) plus soil solarization significantly increased shoot and root dry weights at harvest (225 DAP), improved yield compared to the untreated control, and decreased root-knot gall index of *C. longa* plants compared to the untreated check. Overall, the integration of cultural practices, host resistance, biological control agents, and chemical nematicides can more effectively manage nematode infestations than one tactic alone (Ramana and Eapen, 1999).

Pests of Production: Fungi

Fungal diseases pose a significant threat to turmeric production systems during cultivation and post-harvest. Leaf blotch, leaf spot, and rhizome rot of turmeric pose the greatest economic threat to turmeric producers (Sarma and Anadaraj, 2000).

Leaf blotch infections (causal agent *Taphrina maculans*) may appear in the middle to later parts of the growing season (July-October) (Satyagopal et al., 2014). Though the disease

affects the leaves of the plant, inoculum sources remain in soil and plant debris (Sarma and Anadaraj, 2000). The primary symptom of the disease is brown spots on both the upper- and lower-surface of leaves that ultimately lead to total leaf necrosis (Jayashree et al., 2015). Asci and ascospores are produced on these infected leaves, and the disease can spread rapidly for the rest of the season (Sarma and Anadaraj, 2000). Rhizomes develop poorly as a result of decreased photosynthesis.

Similarly, leaf spot infections (causal agent *Colletotrichum* sp.) result in irregular brown spots on young leaves that fuse to cover the entire leaf surface. Mature spots appear greyish-white surrounded by a yellow halo and produce spore-forming acervuli (Sarma and Anadaraj, 2000). Infected leaves senesce quickly, which was observed to reduce rhizome yield by as much as 62.7% (Jayashree et al., 2015). Both foliar diseases, leaf blotch and leaf spot, are favored by warm, humid conditions (Satyagopal et al., 2014).

Rhizome rot (causal agent *Pythium* spp.) directly affects marketable yield by decaying the rhizomes of both turmeric and ginger (Sarma and Anandaraj, 2000). The disease was first described in turmeric in India, and the pathogen identified as *P. graminicolum* (Ramakrishnan and Sowmini, 1955); however, Sarathi et al. (2014) identified *P. aphanidermatum* as an additional cause of rhizome rot using Koch's postulates. Furthermore, Anoop et al. (2014) found that in addition to *Pythium* spp., *Rhizoctonia* sp. and *Fusarium* sp. were responsible for 6.78% and 5.93%, respectively, of rhizome rot disease incidence across four Indian states. Notably, Eapen et al. (2008) did not observe pathogenic effects of *F. oxysporum* on rhizomes when the fungus was applied as a potential nematode biocontrol. For all causal organisms, infection often initiates toward the beginning or middle of the season (June-September) as young plants are most susceptible to the pathogen (Satyagopal et al., 2014). Upon infection, the pseudostem becomes

water-soaked and structurally compromised, leading to plant collapse and deterioration of rhizomes (Jayashree et al., 2015). *Pythium* spp. and *Fusarium* spp. persist in soil through survival structures and can spread to new areas via seed material (Satyagopal et al., 2014).

Soil-borne fungal pathogens known to infect turmeric including *Pythium*, *Rhizoctonia*, and *Fusarium* species are present throughout cotton-producing regions of Alabama (Gazaway, 1998; Palmateer et al., 2004); therefore, turmeric producers must consider preventative measures and management options for fungal pests.

Current Fungal Management Strategies

Similar to nematode management strategy, a multi-faceted approach is often more effective than a single solution for fungal management. Chemical fungicide use throughout turmeric production in India incorporates a number of different fungicides including (but not limited to) metalaxyl, mercurial compounds, copper oxychloride, propiconazole, and hexoconazole (Shanmugan et al., 2015). Mancozeb applied as a drench can prevent the spread of rhizome rot, and foliar sprays of the fungicide can be used to treat leaf blotch and leaf spot infections (Sarma and Anadaraj, 2000). Repeat applications of fungicides over a season can reduce disease pressure while increasing fresh rhizome yield (Mishra and Pandey, 2015); however, reliance on a single chemical can promote pathogen insensitivity and may eventually render the chemical ineffectual (Brent and Hollomon, 2007). A combination of management tools must be selected to build a more holistic fungal control program.

Cultural practices, as mentioned for nematode control, also aid in fungal management. Katan et al. (1976) found that solarizing soil for fourteen days eliminated 100% of *Verticillium dahlia* inoculum to a depth of 25 centimeters and 54-63% of *Fusarium oxysporum* inoculum at 25 centimeters.

Antagonistic bacteria and fungi, common biological control agents, have proven effective as a rhizome rot management technique. Muthulaskshmi and Saveetha (2009) found that a combination of *Trichoderma viride* and *Pseudomonas fluorescens* applied as a seed treatment and a soil application at planting and 150 DAP reduced rhizome rot disease incidence to less than one percent while increasing yield 30 tonnes per hectare over the control. Similarly, a combination of rhizome treatment, soil application, and top dressing of *P. fluorescens* and *T. harzianum* reduced rhizome rot disease incidence by 79% compared to the control (Shanmugan et al., 2015).

Select varieties of turmeric demonstrate resistance to certain fungal pathogens in India. 'PCT14' and 'Shillong' exhibit resistance to rhizome rot, and 'CLL324', 'Ethamukala', 'CLL316 Gorakhpur', 'CLL326 Mydukur', 'Aleppey', and 'PCT12' show resistance to leaf blotch infections; 'PCT13' was found resistant to both rhizome rot and leaf blotch. 'Bhendi', 'Gadhair', and 'Krishna' are resistant to leaf spot (Sarma and Anadaraj, 2000).

Nematode-Fungi Interaction Observed in *Curcuma* spp.

Sarma (1974) recorded the first observed interaction between nematode and fungal pathogens of *Curcuma* spp.; the association of *Pratylenchus* sp. and *Fusarium* sp. induced stunted growth and exacerbated brown rot (rhizome rot) symptoms on infected rhizomes. In the rhizosphere of ginger, joint inoculation of both fungal and nematode pests revealed *Pythium myriotylum* acts antagonistically towards both the host plant and the nematode pathogen, *M. incognita*; *P. myriotylum* alone caused similar growth reductions to *P. myriotylum-M. incognita* combinations (Lanjewar and Shukla, 1985).

Summary and Research Objectives

In conclusion, turmeric is a globally valuable specialty crop with numerous uses from food coloring to pharmacology. The identification of nutritive qualities of the rhizomes correlates to a rise in demand for turmeric, and fresh market production may prove a worthwhile niche crop in the subtropical climate of Alabama. However, potential growers must be informed of pathogens endemic to regions throughout Alabama that can lead to significant losses in yield. Management tactics for these pests, including chemical, biological, and cultural methods, have proven useful in Indian turmeric production, and a combination of strategies can be implemented to minimize economic losses in Alabama and other southeastern U.S. regions.

The objectives of this study include 1) assessment of the effect of *M. incognita* on the early-season growth of three *C. longa* accessions, 2) evaluation of root-knot nematode susceptibility of eight *C. longa* accessions and potential nematicide management tools, and 3) identification of fungal susceptibility of *C. longa* in Alabama.

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Chapter 2: First Report of the Root-knot Nematode (*Meloidogyne incognita*) on *Curcuma longa* in the United States

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Common turmeric (*Curcuma longa* L.), a spice crop native to India, is found in a variety of products ranging from foods to cosmetics to pharmaceuticals (Prasad and Aggarwal, 2011). The medicinal plant is in early stages of evaluation as niche crop for Alabama; throughout the state, fresh market demand of turmeric is on the rise, particularly in local farmers markets (T. Gonzalez, personal communication). During the summer of 2015, turmeric plants grown on the campus of Auburn University, Auburn, AL, exhibited small to large galls on root systems. Symptoms appeared throughout all eight accessions of *Curcuma longa* grown on the campus of Auburn University. Plants in early summer exhibited symptoms including chlorosis, stunting, and marginal leaf necrosis. Symptomatic plants were collected and root systems exhibited numerous galls, typical of *Meloidogyne* infection. Nematode eggs were extracted from root systems using the NaOCl-extraction method (Hussey and Barker, 1973) and enumerated.

Eggs were hatched to the second juvenile stage (J2) for species identification. Individual juveniles (1-10 per sample) were picked out of the population and placed in a 20 µL droplet of water set on a microscope cover slip. Each juvenile was then smashed into several pieces by a 100 µL pipette tip via the smashing method and immediately used for PCR (Powers and Harris, 1993). The J2 DNA was amplified via PCR using primers IncK-14F and IncK-14R that are specific for amplification of *M. incognita* (Randig et al., 2002). Primers specific for *M. arenaria* (Far/Rar), *M. javanica* (Fjav/Rjav), *M. hapla* (JMV1/JMV hapla), and *M. enterollobi* (Me-F/Me-R) were also used, but failed to amplify any of the unknown nematode DNA (Randig et. al.

2002; Zijlstra et. al. 2000). PCR was run on unknown samples as well as a positive control sample of *Meloidogyne incognita* DNA obtained from the greenhouse stock cultures that have previously been identified as *M. incognita* by this research group (Groover and Lawrence, 2016). Approximately 45-50 J2's were tested with each primer set, and the IncK-14F/IncK-14R primer set amplified about 30 as *M. incognita*, giving an amplification rate around 65%. The amplified PCR product was then run on a 1% agarose gel and a 400 base pair fragment was observed under a UV light, confirming the population to be *M. incognita* (Randig et. al. 2002).

Eggs were used to perform Koch's postulates in a greenhouse pathogenicity test. Three different accessions of turmeric rhizomes ('CL2', 'CL3', and 'CL7') were sown in 15 cm polystyrene pots containing a 1:1 mixture of sand to sterilized soil (Kalmia loamy sand- 80% sand, 10% silt, 10% clay). Each pot containing a turmeric accession was either inoculated when shoots began to emerge near thirty days after planting with 10,000 *Meloidogyne incognita* eggs or not inoculated as a control. The greenhouse in which plants were grown was maintained at 31°C and received fourteen hours of light including both natural and artificial sources. Plants were hand-watered twice each day. All six treatments were replicated five times and arranged in RCBD. The entire test was repeated. Tests were harvested at 60 DAI (days after inoculation). All data were analyzed using SAS 9.4 PROC GLIMMIX, and means were compared using the Tukey-Kramer method with a significance level of $P \leq 0.05$ (Table 1).

At 60 DAI, *M. incognita*-inoculated turmeric accessions CL2 and CL7 exhibited significantly reduced average plant height, shoot fresh weight, and root fresh weight with the measurements being 31%, 50%, and 26% of those of the control, respectively. Inoculated accession CL3 did not present significant differences with the control in terms of plant development. Final nematode population density on CL2, CL3, and CL7 ranged from 19-319

eggs per gram of root, 1-2527, and 41-4703 eggs per gram of root, respectively. Reproductive factor (RF), defined as the final nematode population density divided by the initial inoculum density, was calculated to be 0.6, 4.1, and 2.1 for CL2, CL3, and CL7, respectively. Consequently, turmeric accessions CL3 and CL7 were susceptible to the nematode, as their RF values were greater than 1. Turmeric accession CL2, on the other hand, was somewhat resistant to the nematode as its RF value was less than 1. To our knowledge, this is the first report of *Meloidogyne incognita* infecting *Curcuma longa* in the United States.

Because *M. incognita* has been recorded in 46 out of Alabama's 67 counties, potential growers of turmeric should consider nematode management when developing a holistic integrated pest management plan. As shown in greenhouse testing, turmeric accessions differ in host suitability to *M. incognita* race 3. Variety selection will prove an important step to successful turmeric production in the state of Alabama.

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Table 1. Response of three *Curcuma longa* accessions (*C. longa* 2, *C. longa* 3, *C. longa* 7) with and without *Meloidogyne incognita* inoculum (10,000 eggs and J2) in terms of average plant height (PH), shoot (SFW) and root fresh weight (RFW), total *M. incognita* population density (Total RKN), and reproductive factor (RF), 60 DAI.

Accession, treatment	PH (cm)	SFW (g)	RFW (g)	Total RKN	RF
C. longa 2[y]	41.6 a[z]	26.6 a	55.7 a	0 a	0
C. longa 2, inoculated	29.0 b	13.9 b	49.0 b	5,639 b	0.56
Accession, Treatment	PH (cm)	SFW (g)	RFW (g)	Total RKN	RF
C. longa 3	43.9 a	24.3 a	55.3 a	0 a	0
C. longa 3, inoculated	34.5 a	18.5 a	34.5 a	41,275 b	4.12
Accession, Treatment	PH (cm)	SFW (g)	RFW (g)	Total RKN	RF
C. longa 7	51.7 a	29.4 a	61.3 a	0 a	0
C. longa 7, inoculated	35.0 b	14.0 b	36.5 b	21,228 b	2.12

[y]Comparisons were made only within accessions.
[z]Values followed by the same letter are statistically similar (within accessions). LS-means separated by the Tukey-Kramer method ($P \leq 0.05$).

Chapter 3: First Report of *Rhizoctonia solani* on *Curcuma longa* in the United States

Common turmeric (*Curcuma longa* L.), a spice crop native to India, is found in a variety of products ranging from foods to cosmetics to pharmaceuticals (Prasad and Aggarwal, 2011). The medicinal plant is in early stages of evaluation as niche crop for Alabama; throughout the state, fresh market demand of turmeric is on the rise, particularly in local farmers markets (T. Gonalez, personal communication). During harvest in the 2015 growing season, rhizomes showed symptoms of rhizome rot including disintegrating rhizome tissue, loss of turgidity, and a darker red-brown hue compared to the typical vibrant orange interior rhizome color.

Diseased rhizomes were collected from *Curcuma longa* plants cultivated in the Auburn University Medicinal Garden. Rhizomes were sliced into 0.1-cm thick cross sections and surface sterilized using 95% ETOH for 30 s followed by 0.625% NaOCl for 2 min. Each cross-section was aseptically transferred to an acidified potato dextrose agar (APDA) plate. Cultures were allowed to grow at ambient temperature (23˚C) in an airtight, clear plastic container with 8 hr of light for 3-10 days.

Isolates were identified to species based on reproductive morphology. Fungal cultures were examined at 200-400x under an inverted Nikon eclipse 80i microscope for identifying cultural characteristics. *Rhizoctonia solani* Kühn was identified following the description of Duggar (1915) including the absence of conidia and elongated mycelial cells with near right-angle branching.

Fungal isolates were prepared for DNA extraction by removal of a 3-5 cm plug of each mycelium and placement onto a 10-cm x 10-cm cellophane square placed centrally on a clean APDA plate. After 5-7 days of growth, DNA was extracted from each isolate using a ZR Fungal/Bacterial MiniPrep™ kit from Zymo Research (California, USA). Extracted DNA

concentrations were evaluated using a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA), and extracted DNA products were purified with a Qiagen PCR Purification Kit (Maryland, USA). A 50-µl reaction was prepared for each isolate including 4 µl of each purified DNA sample, 2 µl each of the primers, 18 µl of deionized water, and 24 µl of ReadyTaq Master (Sigma-Aldrich, St. Louis, MO). Reactions (50 µl) were pipetted into 8-tube strips, and PCR was conducted. Two primers, ITS-1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'), were used to differentiate fungal genera. DNA amplification was carried out in a thermocycler as follows: initial denaturation at 94˚C for 5 min followed by 35 cycles of 94˚C for 1 min, 55˚C for 1 min, and 72˚C for 2 min. After amplification, PCR products were sequenced by Eurofins Genomics (Huntsville, AL). Gene sequences were aligned using BioEdit software and analyzed by the Basic Logical Alignment Search Tool (BLAST) nucleotide database. Nine isolates returned a 100% identity to *Rhizoctonia solani*.

Koch's postulates were conducted to affirm the pathogenicity of the identified fungi on *Curcuma longa. Rhizoctonia solani* cultures isolated from diseased rhizomes were transferred from storage tubes to APDA plates using sterile technique and allowed to grow for 5 days. Five mycelial plugs 3 mm in diameter were added to two 500-mL Erlenmeyer flasks containing 200 mL of sterile potato dextrose broth (PDB). After five days of growth, the mycelium was poured into a blender and blended into a slurry.

Curcuma longa rhizomes were sown in 500 cm^3 polystyrene pots in a pasteurized soil sand mix (1:1 v/v) with standard fertilizer and lime. The soil was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from the Plant Breeding Unit of the E.V. Smith Research Center of Auburn University located near Tallassee, AL. Soil was pasteurized at 88˚C for 12 hr

and allowed to cool for 12 hr, and the process was repeated. The plants were housed in the greenhouse at Plant Science Research Center, Auburn, AL. The greenhouse temperature ranged from 22-34°C, and natural light was supplemented with light of 1000-watt halide bulbs producing 110,000 lumens to maintain the day length of 14 hr. Rhizomes were sown on February 1, 2017 and first showed signs of germination mid-March. A 0.4% v/v sterile water and *R. solani* slurry was pipetted onto four *C. longa* plants 14 days after germination to complete the pathogenicity test. The experiment was hand-watered twice each day. The experiment was terminated 60 days after inoculation. Upon termination, all rhizomes exhibited symptoms of rhizome rot including soft texture, disintegrating tissue, and darker coloration. Rhizomes were sliced into 0.1-cm thick cross sections and surface sterilized using 95% ETOH for 30 s followed by 0.625% NaOCl for 2 min.

Each cross-section was transferred to an acidified potato dextrose agar (APDA) plate and allowed to grow for 5 days. Afterward, isolates were aseptically sub-cultured onto new APDA plates and allowed to grow for morphological identification. *Rhizoctonia solani* was successfully re-isolated from the inoculated plants as evidenced by cultures that match the description of Duggar (1915). To our knowledge, this is the first report of *R. solani* infecting *Curcuma longa* in the United States.

Because *R. solani* is an endemic fungal pest to Alabama soils (Palmateer et al., 2004), potential growers of turmeric should consider soil borne-fungus management strategies to minimize economic losses due to rhizome rot.

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Chapter 4: Southern Root-knot Nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) and Fungal Susceptibility of Turmeric (*Curcuma longa* L.) Accessions

Abstract

Curcuma longa L., commonly known as turmeric, has been a staple in Southeast Asian culture, cuisine, and medicine for thousands of years. Demand of turmeric has increased in the United States, and turmeric is undergoing evaluation as a potential cash crop for Alabama.

In the summer of 2015, *C. longa* plants grown on the campus of Auburn University, Auburn, AL, showed symptoms of *Meloidogyne* infection including stunting, chlorosis, and galling on roots. Soil tests revealed the presence of *M. incognita* throughout the turmeric plots. Eight *C. longa* accessions were evaluated for *M. incognita* susceptibility in both greenhouse and microplot trials in the summer and fall of 2016. Growth parameters including plant height, shoot and root fresh weight, and *M. incognita* eggs per gram of root were measured at termination. In both trial settings, *C. longa* 2 and *C. longa* 4 demonstrated taller plant height, and *C. longa* 2 also exhibited greater shoot fresh weight than all other accessions. *Meloidogyne incognita* reproductive factor (RF) for all accessions in the greenhouse experiments exceeded 1.0 (3.9-10.4), indicating all *C. longa* accessions tested are excellent hosts of *M. incognita.* Five chemical nematicides and one biological agent were applied as soil drenches to rhizomes sown in a *M. incognita* infested field. At 90 days after planting, *M. incognita* population density ranged from 34 to 477 eggs per gram of root, and population density of *M. incognita* eggs produced in all nematicide treatments were similar to the untreated control. Turmeric root and shoot weights were also unaffected by the nematicides. Because all turmeric accessions grown on the campus of Auburn University are susceptible to *M. incognita* and nematicides are not an effective method of control, *M. incognita* may prove to be a major pest for Alabama turmeric production.

In addition to *M. incognita* infestation, rhizomes harvested from the turmeric plots in January 2016 displayed symptoms of rhizome rot (causal agents *Pythium* spp., *Rhizoctonia* sp., *Fusarium* sp.) including discoloration and macerated texture. Diseased rhizomes from each accession were cultured, and fungal DNA was extracted from the isolates. Sequences were amplified by PCR for identification using fungal-specific primers ITS-1 and ITS-4. Forty-one sequences were aligned and matched to genus and 15 were matched to a singular species. Genera included *Fusarium* spp., *Rhizoctonia solani*, *Trichoderma* spp., *Macrophomina phaseolina*, *Diplodia seriata*, and *Nigrospora* sp. The most frequently isolated fungi were *Fusarium* (60.9%) and *Rhizoctonia solani* (21.9%). Koch's postulates, a method of establishing a causative relationship between an organism and a pathogen, was completed for *R. solani* on *C. longa*.

Introduction

Turmeric (*Curcuma longa* L.), a spice crop native to Southeast Asia (Prasad and Aggarwal, 2011), has experienced a dramatic increase in demand due to its anti-inflammatory and other health-promoting properties (Daily et al., 2016; Chuengsamarn et al., 2012; Zheng et al., 2016). Many health attributes of turmeric have been attributed to curcumin, the chemical constituent that separates *C. longa* from other species of the Zingiberaceae family; however, additional compounds present in rhizomes including turmerones, elemene, furanodiene, cyclocurcumin, and germacrone also demonstrate antioxidant and/or anti-cancerous activity (Aggarwal et al., 2013). The subtropical environment and sandy soil types of central and southern Alabama may provide a similar production scenario to the spice's native land; therefore, turmeric is undergoing evaluation as a potential cash crop for Alabama and other Southeastern U.S. states (T. Gonzalez, personal communication).

Thirteen accessions of turmeric (*Curcuma* spp.) are cultivated on the campus of Auburn University, Auburn, AL as shown in Fig. 1A and B, and plots are maintained by the manager of the Auburn University Medicinal Garden.

Fig. 1. The Medicinal Plant Collection located at Auburn University, Auburn, AL (A) and a mature *Curcuma longa* plant in the Medicinal Plant Collection (*C. longa* 5) (B).

A. 

B. 

Turmeric plots were located adjacent to the Medicinal Plant Collection on ground previously cropped with sugarcane. The turmeric plots were located directly next to a field formerly used for nematode (including *Meloidogyne*) trials, and the general area of the Auburn University Medicinal Garden including the turmeric plots is historically known to be infested with *Meloidogyne* (D. Delaney, personal communication). The soil type in this area is a Marvyn loamy sand (81% sand, 15% silt, 4% clay), and sandy soils are conducive to *Meloidogyne*. The seed material, turmeric rhizomes, are typically sown in May and harvested in late December through early January. Rhizomes from the previous season are used as propagation material for the following season. After harvest, rhizomes as shown in Fig. 2A were washed and left to dry on brown paper at ambient temperature (21°C) for one week as shown in Fig. 2B to reduce moisture before being stored in open paper bags at ambient temperature to decrease the risk of storage rots.

Fig. 2. Rhizomes harvested from the turmeric plots adjacent to the Auburn Medicinal Plant Collection (A) and harvested rhizomes drying at the Plant Science Research Center, Auburn, AL (B).

A.  B. 

Rhizomes not kept for seed material are distributed to small organic and sustainable farms throughout the state and greater parts of the southeastern U.S. seeking a niche crop for fresh market production.

Meloidogyne **Susceptibility of** ***Curcuma longa***

In the summer of 2015, plants from all of the accessions showed symptoms of *Meloidogyne* infection including stunting, chlorosis, and galling on root systems as shown in Fig. 3.

Fig. 3. Galling on *Curcuma longa* feeder roots, indicative of *Meloidogyne* infection.

Foliar and root damage was present throughout the turmeric plots, though no quantitative data (plant height, dry or fresh shoot or root weight, etc.) was taken other than rhizome harvest data in December-January of 2015-2016. To prevent dispersal of *Meloidogyne* by infested rhizomes as described by Cheng and Tu (1979), the turmeric plots were moved to a different site on the agronomy farm at Auburn University the following season. *Meloidogyne* spp. are classified as endemic pests throughout the United States (Thomas and Kirkpatrick, 2001) and are present in Alabama (Walters and Barker, 1994); therefore, the root-knot nematode may pose a significant threat to Alabama turmeric production.

The genus *Meloidogyne* includes over 90 species, but four species of *Meloidogyne; M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* Treub deserve mention due to their worldwide distribution and economic importance. *Meloidogyne arenaria, M. incognita,* and *M. javanica* affect a wide range of monocotyledon and dicotyledon plants especially in regions with longer growing seasons (Hunt and Handoo, 2009). *Meloidogyne javanica* is common closer to the equator (35°S-30°N), and *M. incognita* and *M. arenaria* span further north to 35°N, often coexisting within similar regions. *Meloidogyne hapla,* often called the northern root-knot nematode, is more commonly found parasitizing dicotyledons in regions with an average temperature of 0°C in winter and 15°C in the summer (Taylor and Sasser, 1978). *Meloidogyne incognita* and *M. arenaria* are the dominant species present in Alabama (Groover and Lawrence, 2016), and both species can be further differentiated into races based on host range. Sasser (1972) described the host ranges of races using the schematic as shown in Fig. 4. *Meloidogyne incognita* race 3 is the foremost species and race found in Alabama soils (Groover and Lawrence, 2016).

Fig. 4. North Carolina differential host test as described by Sasser (1972).

Meloidogyne Species and Race	Cotton 'Deltapine 61'	Tobacco 'NC95'	Pepper 'California wonder'	Watermelon 'Charleston Gray'	Peanut 'Florunner'	Tomato 'Rutgers'
M. incognita						
race 1	-	-	+	+	-	+
race 2	-	+	+	+	-	+
race 3	+	-	+	+	-	+
race 4	+	+	+	+	-	+
M. javanica	-	+	-	+	-	+
M. arenaria						
race 1	-	+	+	+	+	+
race 2	-	+	-	+	-	+
M. hapla	-	+	+	-	+	+

Meloidogyne spp. can damage a vast range of plants, including members of the ginger family (Koshy and Bridge, 1990). *Meloidogyne incognita* negatively affects turmeric production systems, causing significant losses to production each year. In the Indian state Kerala, yield loss in *Meloidogyne*-infested fields reached as high as 45.3% (Bai et al., 1995). Ray et al. (1995) found that the presence of *M. incognita* reduced yield in Indian turmeric and ginger crops by 33.6% and 26.3%, respectively. Haider et al. (1998) reported 1,000 *M. incognita* J2 nematodes per 500 grams of soil reduced leaf area, shoot length, shoot weight, root weight, and rhizome weight compared to a non-inoculated control, and as little as 100 *M. incognita* J2 per 500 grams of soil reduced rhizome weight compared to the control.

Meloidogyne infestation symptoms include stunting, chlorosis of leaves, and poor shoot development (Greco and Di Vito, 2009). The infective-stage juveniles (J2) enter fibrous roots through the point of differentiation but can move into fleshy roots along the entire length of the root; additionally, the axils of leaf sheaths in the shoot apex of a newly germinated rhizome present another point of entry to invading J2 (Cheng and Tu, 1979). Infection by any *Meloidogyne* species results in galling on roots caused by internal root feeding that induces plant

cell hypertrophy (cell enlargement) and hyperplasia (increase in cell number). Infected rhizomes will exhibit a dull yellow color (Ramana and Eapen, 1999). Plants inoculated with the root-knot nematode exhibited the aforementioned symptoms as well as marginal leaf necrosis and a reduced number of shoots compared to control plants after six months (Ramana and Eapen, 1995). The life cycle of *M. incognita* spans thirty days on turmeric plants at temperatures of 25-35˚C (Mohanta and Swain, 2014a); therefore, a typical nine-month growing season of the crop in the southern United States can see as many as nine generations of the nematode pest.

Current *Meloidogyne* Management Strategies

Variety selection is a crucial step in the production process of any crop, particularly when a pathogen is present and host resistance is available. Nematode resistance is available in select varieties of turmeric. Indian varieties Armoor, Duggirala, Guntur-1, Guntur-9, Rajampet, Sungandham, and Upplapadu were observed to demonstrate resistance to the root-knot nematode and are recommended in areas of high infestation (Ramana and Eapen, 1999). Mohanta et al. (2015) reported less galling on *Meloidogyne*-inoculated rhizomes of cultivars 'Durigala', 'PTS-31', 'Ansitapani', 'PTS-21', and 'PTS-47', leading to the classification of these cultivars as resistant to the root-knot nematode.

Additional management strategies including chemical nematicides and insecticides can efficiently decrease nematode population density; however, turmeric is an experimental, high value, small acreage crop for the U.S., thus no nematicides are registered for use on this crop. Carbofuran™ has been reported to reduce *Meloidogyne* population density in Indian turmeric production by as much as 81.6% (Ramana and Eapen, 1999), but the chemical was classified as ineligible for reregistration in the United States by the Environmental Protection Agency (EPA) in 2007 (EPA, 2007). Additionally, methyl bromide, which was often used on specialty high

value crops, was phased out of U.S. crop production in 2005 (EPA, 2016). Bayer CropScience, DuPont, and Marrone Bio Innovations have recently brought new nematicides to the market for vegetable crops; however, none have been registered for turmeric production systems. The introduction of turmeric into production systems of the U.S. opens up opportunity for exploration of new production systems for this crop with new nematicidal options that are both effective and sustainable.

Objectives of nematode susceptibility studies include: 1) evaluation of *C. longa* accessions for susceptibility to *M. incognita* during early season growth and 2) assessment of chemical nematicides in the field as a potential *M. incognita* management strategy.

Fungal Susceptibility

During the rhizome harvest in the 2015 growing season and continuing today, diseased rhizomes are present throughout the turmeric plots. Symptoms include disintegrating rhizome tissue resulting in loss of turgidity and a darker red-brown hue compared to the typical vibrant orange interior rhizome color.

Rhizome rot (causal agent *Pythium* spp., *Rhizoctonia* sp., *Fusarium* sp.) directly affects marketable yield by decaying the rhizomes of both turmeric and ginger (Sarma and Anandaraj, 2000). The disease was first described in turmeric in India, and the pathogen identified as *P. graminicolum* (Ramakrishnan and Sowmini, 1955); however, a study conducted by the Indian Institute of Spices Research in 2006-2007 found that in 188 samples for 38 locations, *Rhizoctonia* was present in 30.5% of symptomatic rhizome rot samples, and *Fusarium* was found in 27.1% (IISR, 2007). Post-harvest losses during storage due to rhizome rot can reach as high as 50-80% (Sarathi et al., 2014).

Infection often initiates toward the beginning or middle of the season (June-September) when young plants are most susceptible to the pathogen (Satyagopal et al., 2014). Upon infection, the pseudostem becomes water-soaked and structurally compromised, leading to plant collapse and deterioration of rhizomes (Jayashree et al., 2015).

The objectives of fungal susceptibility evaluation include: 1) isolation of the fungal pathogens colonizing the diseased rhizomes, 2) identification of the fungal species present in diseased rhizomes both molecularly and morphologically, and 3) completion of Koch's postulates to affirm the pathogenicity of the causal agent(s).

Materials and Methods

Meloidogyne incognita Susceptibility

Accessions of the Auburn University Medicinal Plant Collection

The turmeric plots located adjacent to the Medicinal Plant Garden on the campus of Auburn University, Auburn, AL, included thirteen *Curcuma* spp. during the 2014 and 2015 growing seasons (Table 1). Upon reception by the Auburn University Medicinal Plant Collection, the seed material was assigned a number by the manager of the Auburn University Medicinal Garden designating seed from the same source and date of addition to the collection. For the purpose of this research, only species identified as *C. longa,* known as common turmeric, were evaluated due to limitations in space and number of rhizomes available.

Field soil sampling for plant-parasitic nematodes

Turmeric accession plots (0.6-m by 1.8-m) directly next to the Auburn University Medicinal Garden, Auburn, AL were sub-sectioned into four subplots per accession. Samples were taken February 24, 2016 shortly after rhizome harvest (2015-2016 season). One bulk soil sample was collected from each subplot by taking eight 2.5-cm dia x 20-cm deep soil samples,

mixing the soil, and processing a 150 cm^3 subsample for nematode extraction. Accession plots were planted in the same location each year. Vermiform nematodes were extracted from each soil sample using the gravity sieving and sucrose-centrifugation methods (Jenkins, 1964). *Meloidogyne* J2 were identified and enumerated at 40x magnification using an inverted TS100 Nikon microscope.

Greenhouse accession trial

The greenhouse trials were conducted at the Plant Science Research Center (PSRC), Auburn University, AL. The soil was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from the Plant Breeding Unit of the E.V. Smith Research Center of Auburn University located near Tallassee, AL. Soil was pasteurized at 88˚C for 12 hr and allowed to cool for 12 h, and the process was repeated.

Meloidogyne incognita, originally isolated from an infested field at the Plant Breeding Unit (PBU) at E. V. Smith Research Center of Auburn University and maintained on corn plants "Mycogen 2H723" (Dow AgroScience, Indianapolis, IN) in 500 cm^3 polystyrene pots in the greenhouse, was used as inoculum in the experiments. Eggs were extracted from corn roots by a modification of the standard technique described by Hussey and Barker (1973) by placing the root system in a 0.625 % NaOCl solution and agitating the roots for 4 min using a rotary shaker at 120 rpm. Eggs were rinsed with tap water, collected on a 25-µm-pore sieve, then processed by sucrose centrifugation-flotation at 240 g for 1 min. Eggs were enumerated at 40x magnification using an inverted TS100 Nikon microscope and standardized to 2,000 eggs per pot for greenhouse tests.

Accessions included *Curcuma longa* 2, *C. longa* 3, *C. longa* 4, *C. longa* 5, *C. longa* 6, *C. longa* 7, *C. longa* 8, and *C. longa* 9 rhizomes from the 2015-2016 growing season. The eight

accessions of turmeric rhizomes were sown in 500 cm^3 polystyrene pots in a pasteurized soil sand mix (50:50 v/v) with standard fertilizer and lime. One rhizome per pot was planted in the soil to a depth of 5 cm. After one month, when approximately 50% of the rhizomes had sprouted, 1.5 ml of water containing 2,000 *M. incognita* eggs was pipetted into a 5-cm depression adjacent to each rhizome, and the depression covered with soil from the same pot. Experiments were arranged in a randomized complete block design (RCBD). Each accession had five replications and the test was repeated. The greenhouse temperature ranged from 9-37°C, and natural light was supplemented with light of 1000 watt halide bulbs producing 110,000 lumens to maintain the day length of 14 hr. The experiment was hand-watered twice each day. Sixty days after inoculation (DAI), plant growth measurements and nematode population density were determined. Plant growth measurements included plant height, shoot fresh weight, and root fresh weight. Nematode population density was measured both per gram of root and total nematode population recovered from roots. Reproductive factor was ascertained for each accession by dividing the final nematode population density by the initial inoculum population density (Oostenbrink, 1966).

Microplot accession trial

Eight *C. longa* accessions were sown in 26.5-l pot-in-pot design microplots located at the Plant Science Research Center, Auburn University, AL. Accessions were the same used in the greenhouse test. Microplots were filled with a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from the Plant Breeding Unit of the E.V. Smith Research Center of Auburn University located near Tallassee, AL. The microplots contained a detectable population of *M. incognita* race 3, and one half of an inoculum pot (250 cm^3 of soil and roots per half pot) containing an average population of 50,000 *M. incognita* eggs and J2 per half pot was placed in

each microplot at planting. Two sprouted rhizomes were sown in each microplot by planting the rhizomes 5 cm into the soil. Accessions were arranged in a RCBD with five replications of each accession. Microplots were irrigated by an automated drip irrigation system set to run for 15-45 min twice a day to administer 450-1350 ml of water per day. At 60 DAP, plants were harvested from each microplot and evaluated for plant height, shoot fresh weight, root fresh weight, and nematode population density in terms of eggs per gram of root and total population density extracted from roots using the method described by Hussey and Barker (1973). The test was first conducted in May-July and was repeated in July-September.

Chemical nematicide field trial

Six chemical nematicidal products (five synthetic products and one biological control agent) were evaluated in field trials for their effect on *M. incognita* population development and plant growth promotion in turmeric. Trials were conducted at the Brewton Agricultural Research Unit, Brewton, AL. The field soil type is a Benndale fine sandy loam (73% sand, 20% silt, 7% clay) and the area is naturally infested with *M. incognita*. Additional inoculum was added at planting by adding one inoculum pot (500 $cm^3$of soil and roots per pot) containing an average population of 100,000 *M. incognita* eggs and J2 per pot to the furrow of each plot at planting. Chemicals applied included Velum Total™ (fluopyram + imidacloprid, 1.25 l/ha, Bayer Crop Science, Raleigh, NC), Counter™ (terbufos, 6 oz./1000 row feet, AMVAC Chemical Corporation, Newport Beach, CA), Avicta™ (abamectin, 1.2 l/ha, Syngenta, Greensboro, NC), Vydate-L™ (oxamyl, 47 l/ha, DuPont, Wilmington, DE), Movento™ (spirotetramat, 0.03 l/ha, Bayer Crop Science, Raleigh, NC), and Majestene™ (*Burkholderia* sp., strain A396, 18.9 l/ha, Marrone Bio Innovations, Davis, CA). An untreated control was also included. None of the nematicides are currently labeled for use on turmeric, however they are registered for use to

manage nematodes on other field and/or vegetable crops in the U.S. Field plots were planted in one-row beds, 1.2 m long with 0.5-m row spacing. Four *C. longa* 2 rhizomes were sown 0.3 m apart in each plot. Nematicide treatments were applied as soil drenches at planting by mixing appropriate rates into 3.8-l watering cans and pouring 0.95 l of chemical onto each plot. The experiments were arranged in a RCBD with four replications and the test was repeated. Plants were watered as needed by an overhead sprinkler irrigation system. Tests were terminated 90 DAP. Plots were harvested and parameters measured included plant height, shoot and root fresh weight, and nematode population density in terms of eggs per gram of root and total *M. incognita* population density extracted from roots.

Statistical analysis

Data from field soil sampling, greenhouse and microplot experiments, and chemical nematicide field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Dependent variables included *M. incognita* J2 per 150 cm^3 of soil (field soil sampling only), plant height (PH), shoot (SFW) and root fresh weight (RFW), *M. incognita* eggs per gram of root, and total *M. incognita* population density. Random effects included replication and test repeat. Student panels were produced to determine the normality of the residuals. LS-means of the growth parameters listed above were compared among accessions by the Tukey-Kramer method for field soil sampling and greenhouse and microplot trials. Dunnett's method was used to compare treatments to an untreated control for chemical nematicide field tests. A significance value of $P \leq 0.05$ was used for the soil sampling data, and a significance value of $P \leq 0.10$ was assigned to the greenhouse, microplot, and field tests due to greater variability within the data sets.

Fungal Susceptibility

Morphological identification

Fungal cultures were examined at 200-400x under an inverted Nikon eclipse 80i microscope for identifying cultural characteristics.

DNA extraction and isolate identification

Diseased rhizomes were collected from each of the thirteen accessions of *Curcuma* spp. cultivated in the Auburn University Medicinal Garden. Rhizomes were sliced into 0.1-cm thick cross sections and surface sterilized using 95% ETOH for 30 s followed by 0.625% NaOCl for 2 min. Each cross-section was aseptically transferred to an acidified potato dextrose agar (APDA) plate. Cultures were allowed to grow at ambient temperature (23˚C) in an airtight, clear plastic container with 8 hr of light for 3-10 days. Fungal colonies emerging from the tissues were recorded, and more than one fungus was often isolated from a single piece of plant tissue. Isolates were identified to morphologically and molecularly identified to species.

Fungal isolates were prepared for DNA extraction by removal of a 3 mm plug of each mycelium and placement onto a 10-cm x 10-cm cellophane square placed centrally on a clean APDA plate. After 5-7 days of growth, DNA was extracted from each isolate using a ZR Fungal/Bacterial MiniPrep™ kit from Zymo Research (California, USA). Extracted DNA concentrations were evaluated using a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA), and extracted DNA products were purified with a Qiagen PCR Purification Kit (Maryland, USA). A 50-µl reaction was prepared for each isolate including 4 µl of each purified DNA sample, 2 µl each of the primers, 18 µl of deionized water, and 24 µl of ReadyTaq Master (Sigma-Aldrich, St. Louis, MO). Reactions were pipetted into 8-tube strips and PCR was conducted. Two primers, ITS-1 (5’- TCCGTAGGTGAACCTGCGG-3’) and ITS-4 (5’-

TCCTCCGCTTATTGATATGC-3'), were used to differentiate fungal genera; these two primers are commonly used for distinguishing fungi based on the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) (Martin and Rygiewicz, 2005). DNA amplification was carried out in a thermocycler as follows: initial denaturation at 94˚C for 5 min followed by 35 cycles of 94˚C for 1 min, 55˚C for 1 min, and 72˚C for 2 min. After amplification, PCR products were sequenced by Eurofins Genomics (Huntsville, AL). Gene sequences were aligned using BioEdit software and analyzed by the Basic Logical Alignment Search Tool (BLAST) nucleotide database.

Statistical analysis

Mean isolation frequency (MIF) was calculated for each genus of fungi as follows, with all frequencies expressed as percentages:

MIF = [Number of isolates of a genus/Total number of samples]*100

Koch's postulates

Koch's postulates were conducted to affirm the pathogenicity of identified fungi on *Curcuma longa*. Limitations in seed material and greenhouse space led to the selection of a single identified fungus for pathogenicity testing. Eapen et al. (2008) did not observe pathogenic effects of *F. oxysporum* on rhizomes when the fungus was applied as a potential nematode biocontrol; therefore, this fungus was not selected for completing Koch's postulates. Additionally, Muthulaskshmi and Saveetha (2009) found that *Trichoderma* can be used as an antagonistic biocontrol agent for rhizome rot of turmeric, and *Trichoderma* was not assumed pathogenic to *C. longa*. *Rhizoctonia solani* emerged as the most common, previously identified pathogen on turmeric present in the turmeric plots and was therefore selected for pathogenicity testing.

Rhizoctonia solani cultures isolated from diseased rhizomes were transferred from storage tubes to APDA plates aseptically and allowed to grow for 5 days. Five mycelial plugs three mm in diameter were added to two sterile 500-ml Erlenmeyer flasks containing 200 ml of sterile potato dextrose broth (PDB). After five days of growth, the mycelium was poured into a blender and blended into a complete slurry.

Four *C. longa* rhizomes purchased at Publix Supermarket, in Auburn, AL were sown in 500 cm^3 polystyrene pots in a pasteurized soil sand mix (1:1 v/v) with standard fertilizer and lime. The soil was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from the Plant Breeding Unit of the E.V. Smith Research Center of Auburn University located near Tallassee, AL. Soil was pasteurized at 88˚C for 12 hr and allowed to cool for 12 hr, and the process was repeated. The plants were housed in the greenhouse at Plant Science Research Center, Auburn, AL. The greenhouse temperature ranged from 22-34°C, and natural light was supplemented with light of 1000-watt halide bulbs producing 110,000 lumens to maintain the day length of 14 hr. Rhizomes were sown on February 1, 2017 and first showed signs of germination mid-March. A 0.4% v/v sterile water and *R. solani* slurry was pipetted onto four *C. longa* plants 14 days after germination to initiate the pathogenicity test. The experiment was hand-watered twice each day. The experiment was terminated 60 days after inoculation. Upon termination, rhizomes were sliced into 0.1-cm thick cross sections and surface sterilized using 95% ETOH for 30 s followed by 0.625% NaOCl for 2 min. Each cross-section was transferred to an acidified potato dextrose agar (APDA) plate and allowed to grow for 5 days. Afterward, isolates were aseptically sub-cultured onto new APDA plates and allowed to grow for morphological identification.

Results and Discussion

Meloidogyne incognita Susceptibility

Field soil sampling

Each accession located at the turmeric plots of the Auburn University Medicinal Garden supported a *Meloidogyne* population. *Meloidogyne* second-stage juvenile nematodes extracted from plot soil samples ranged from 141 to 1284 J2 per 150 cm^3 of soil. *Curcuma longa* 5 (originally from India) contained the highest population density of J2 ($P \leq 0.05$) while all other accessions displayed statistical similarity of J2 population density (Table 2).

Meloidogyne spp. are considered one of the most common and damaging nematode pathogens, and *M. incognita* is identified as the most widespread of the *Meloidogyne* species (Trudgill and Blok, 2001). *Meloidogyne incognita* race 3 was identified as the most prevalent species in Alabama (Groover and Lawrence, 2016) and was therefore used in further *C. longa* susceptibility experimentation. Nematode population density for each accession exceeded the recommended soil threshold for root-knot nematode (>10 nematodes per 100 cm^3 of soil during spring) as recommended by the University of Georgia Cooperative Extension Service (Davis et al., 2001). Growers who choose to cultivate turmeric in infested areas must consider nematode management when formulating an integrated pest management program.

Greenhouse and microplot accession trials

Accessions responded differently to *M. incognita* infestation. In the greenhouse, *C. longa* 2 grew taller than *C. longa* 5, *C. longa* 6, and *C. longa* 8 ($P \leq 0.1$) by an average of 11 cm and had a similar plant height to the accessions *C. longa* 3, *C. longa* 4, *C. longa* 7 and *C. longa* 9 (Table 3). Additionally, the shoot fresh weight of *C. longa* 2 was greater than all other accessions ($P \leq 0.1$). *Curcuma longa* 7 had greater root fresh weight compared to all other accessions

($P \leq 0.1$). *Meloidogyne incognita* eggs extracted from the greenhouse turmeric accessions ranged from 196-1489 eggs per gram of root; *C. longa* 4 contained lower population density of *M. incognita* eggs than *C. longa* 9 ($P \leq 0.1$). Reproductive factor of *C. longa* accessions ranged from 3.9-10.4, with *C. longa* 4 exhibiting the lowest RF.

Turmeric accessions differed in plant growth and *M. incognita* infestation in microplot testing. *Curcuma longa* 2 and *C. longa* 4 demonstrated tallest plant height ($P \leq 0.1$) (Table 4). The shoot fresh weight of *C. longa* 2 was also greater than all other accessions ($P \leq 0.1$). *Curcuma longa* 2, *C. longa* 4, and *C. longa* 7 exhibited greater root fresh weight than all other accessions ($P \leq 0.1$). *Meloidogyne incognita* eggs extracted from the microplot experiment ranged from 301-1505 eggs per gram of root. *Curcuma longa* 2 supported a lower number of eggs per gram of root than *C. longa* 3, *C. longa* 5, and *C. longa* 6; *C. longa* 4 sustained fewer eggs per gram of root than *C. longa* 3 and *C. longa* 5 ($P \leq 0.1$). Total *M. incognita* population density ranged from 22,920-42,387 across accessions with all accessions demonstrating statistical similarity.

In both the greenhouse and microplot trials, *C. longa* 2 and *C. longa* 4 emerged as accessions exhibiting either increased plant growth, decreased nematode population density, or both. In a supplementary greenhouse study in which *C. longa* 2, *C. longa* 3, and *C. longa* 7 were inoculated with 10,000 *M. incognita* eggs and J2, the reproductive factor of *C. longa* 2 indicated this accession was not a susceptible host (Hall et al., 2017). *Curcuma longa* 2 and *C. longa* 4 were observed to possess lower concentrations of curcumin, the most widely recognized and sought after chemical constituent of the rhizome in terms of nutraceutical properties (D. Shannon, unpublished data). Turmeric varieties with greater concentrations of curcumin typically contain 4% or more curcumin per dry weight (Lal, 2012), and though published research is limited, these varieties are often associated with both fungal and *M. incognita* resistance in

Indian turmeric production (Chand and Pandy, 2014; Rao et al., 1994). Lower curcumin content is not associated with nematode resistance in the current literature. However, curcumin is only one of the many phytochemicals present in turmeric rhizomes (Aggarwal et al., 2013); another compound may be responsible for decreasing susceptibility of these accessions. Quality characteristics of turmeric including curcumin, essential oil, and oleoresin content negatively correlate with yield (Peter et al., 2007). Although *C. longa* 2 and *C. longa* 4 are considered low in curcumin, greenhouse and microplot testing revealed these accessions performed well in development in the first 60 days of growth as well as full-season yield (D. Shannon, unpublished data). The proliferative growth of these accessions may provide an edge over slower growing accessions. These accessions can provide valuable, higher-yielding genetic material for turmeric breeding programs.

Chemical nematicide field trial

Chemical nematicide treatments in the field generally did not differ from the untreated control in terms of plant growth (Table 5). No phytotoxicity was observed throughout testing. Further experimentation should be conducted to establish an effective chemical and/or biological product application as a part of an integrated pest management plan. Biological control agents have proven to be an effective nematode management strategy. Eapen et al. (2008) investigated various fungal bioagents including *Aspergillus* sp., *Fusarium oxysporum*, and *Paecilomyces lilacinus* as a means of nematode control. All fungal bioagents increased plant height compared to the control, and *F. oxysporum* increased rhizome yield (40.9% increase over control) in field trials. Mohanta and Swain (2014b) found that combinations of integrated pest management including crustacean exoskeleton amendment (0.75 tonne/ha) plus soil solarization, carbofuran (0.75 kg a.i./ha) plus *Bacillus subtilis* in aqueous solution (5 L/ha), and sesame oil cake (0.75

tonne/ha) plus soil solarization increased shoot and root dry weights at harvest (225 DAP), improved yield compared to the untreated control, and decreased root-knot gall index of *C. longa* plants compared to the untreated check. Overall, the integration of cultural practices, host resistance, biological control agents, and chemical nematicides can more effectively manage nematode infestations than one tactic alone (Ramana and Eapen, 1999); therefore, future studies may evaluate these techniques both singly and in combination with each other to discover which practices offer the most effective management of *M. incognita*.

Fungal Susceptibility

Morphological identification

Fungal cultures examined under magnification revealed the presence of six fungal genera. *Fusarium* Link. was distinguished by pink-purple hues intermingled with extensive, cotton-like mycelium, hyaline conidia, canoe-shaped macroconidia, and single-celled, ovoid or oblong microconidia (Nelson et al., 1994). *Trichoderma* Pers. was identified by rapidly growing, green mycelium, non-verticillate, highly branched conidiophores, and terminally borne single-celled conidia (Jaklitsch et al., 2006). *Diplodia* Fr. exhibited black, globose pycnidia with slender conidiophores bearing two-celled, dark conidia (Phillips et al., 2007). *Nigrospora* Zimm. was characterized by short conidiophores with shiny, black, single-celled conidia borne on hyaline vesicles at the end of each conidiophore (Mason, 1927). *Rhizoctonia* Kühn (Corticum Vagum B & C) was identified by the absence of conidia and elongated mycelial cells with near right-angle branching (Duggar, 1915). *Macrophomina* (Tassi) Goid exhibited visible, dark microsclerotia (Holliday and Punithalingam, 1970).

DNA extraction and isolate identification

Though fungal isolates were obtained from all accessions, only 55 isolates extracted from *Curcuma aeruginosa*, *C. amada*, *C. caesia* 2, *C. caesia* 3, *C. longa* 2, *C. longa* 3, *C. longa* 4, *C. longa* 6, *C. longa* 7, *C. longa* 9, and *C. zeodaria* were submitted for sequencing due to morphologically similarity of cultures across accessions and cost. Of the 55 isolates submitted to Eurofin Genomics for sequencing, 41 sequences were clearly aligned (Table 6). The analysis revealed six genera including *Fusarium* (MIF=60.9%), *Rhizoctonia* (MIF=21.9%), *Trichoderma* (MIF=9.7%), *Macrophomina* (MIF=2.5%), *Diplodia* (MIF=2.5%), and *Nigrospora* (MIF=2.5%) (Table 7). *Fusarium* spp., *Rhizoctonia solani*, *Trichoderma* spp., *Macrophomina phaseolina*, and *Nigrospora oryzae* have been observed throughout Alabama (Palmateer et al., 2004); *Diplodia* is the only genus previously unidentified in an Alabama production system.

Of the *Fusarium* spp., ten isolates were matched with 100% identity to a single species. *Fusarium* spp. included seven *F. oxysporum,* two *F. verticillioides*, and one *F. sacchari* isolate. *Fusarium oxysporum* f. sp. *vasinfectum* (Armstrong and Armstrong, 1960) is the causal agent of Fusarium wilt in upland cotton, a commodity common in Alabama, but *F. oxysporum* was not observed to be pathogenic on *Curcuma longa* (Eapen et al., 2008). Septiana et al. (2017) found both *F. oxysporum* and *F. verticillioides* as endophytes within turmeric plants; endophytic fungi live inside plants, often providing some type of benefit to the host (Dai et al., 2008). Though *F. sacchari* has previously not been reported on turmeric, the literature suggests *Fusarium* spp. generally live symbiotically rather than parasitically on turmeric.

Nine isolates returned 100% matches to *Ceratobasidium* sp. and *Rhizoctonia* sp., a teleomorph (sexual)-anamorph (asexual) complex, respectively (Oberwinkler et al., 2013); however, the lack of basidial morphology when viewed under a microscope indicate these

isolates belong to the asexual classification. *Rhizoctonia solani* infects over 500 plant species as a necrotropic parasite (Farr et al., 1995) and is a common pathogen of cotton and green beans in Alabama (Palmateer et al., 2004; Sikora and Kemble, 2004). The fungus has been identified in Indian turmeric production as a contributor to rhizome rot (Anoop et al., 2014) and has also been identified on turmeric leaves, inciting leaf blight (Roy, 1992). The prevalence of identified *R. solani* isolates from the Auburn University turmeric plots (21.9% of all isolates) and the pathogenic nature of this pest suggest this fungus may be problematic for Alabama turmeric growers.

One isolate returned a 100% match to *Macrophomina phaseolina*, the causal agent of charcoal rot of soybeans. While the fungus is commonly found in legume-cultivated Alabama soils (Wyllie, 1988), *M. phaseolina* has also been documented to infect medicinal plants in India (Lodha et al., 1986). Kumar and Roy (1990) observed *Fusarium* spp., *R. solani*, and *M. phaseolina* associated with storage rots of turmeric rhizomes in Indian production systems.

Two *Trichoderma* spp., *T. virens* and *T. gamsii*, returned a singular 100% identity match. *Trichoderma* has not been cited as pathogenic on turmeric throughout the literature. *Trichoderma* is largely regarded as a beneficial fungal genus; research has revealed that *T. virens* promotes plant growth and *T. gamsii* exhibits antagonism against phytopathogenic fungi (Contreras-Cornejo et al., 2009; Rinu et al., 2014). The manager of the turmeric plots applied biological product RootShield Plus™ (ARBICO Organics, Oro Valley, AZ, active ingredients *T. harzianum* Rifai strain T-22 and *T. virens* G-41) to the plots during the summer of 2013 for fungal control and plant growth promotion (T. Gonzalez, personal communication).

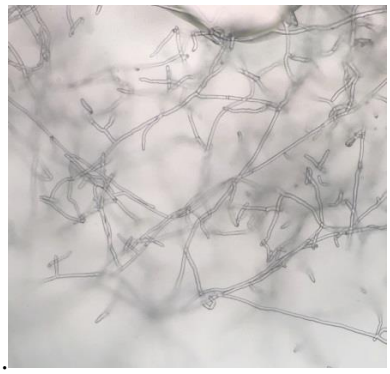
Nigrospora sp. and *Diplodia seriata* have not been associated with turmeric in the current literature. Sugarcane (*Saccharum* sp.), the previous crop of the Auburn University turmeric plots,

is a known host of *Nigrospora oryzae* (Farr et al., 1995). The fungus is present in Alabama and has been cited as the causal agent of lint rot on cotton bowls in southern parts of the state (Palmateer et al., 2003); however, Palmateer et al. (2004) observed *N. oryzae* in only 6.5% of total diseased samples across six Alabama locations (2000-2001). Additionally, *D. seriata* does not exhibit a strong pathogenic in presence in Alabama. Typical hosts of *D. seriata* such as apple (*Malus* sp.) and grape (V*itis vinifera*) (Phillips et al., 2007) are not widely cultivated in Alabama due to disease pressure and unsuitable environmental conditions. These fungi did not exhibit a strong presence throughout the Auburn University turmeric plots as only one isolate of each fungus was identified.

Koch's postulates

At termination 60 DAI, all rhizomes exhibited symptoms of rhizome rot including soft texture, disintegrating tissue, and darker coloration. *Rhizoctonia solani* was successfully re-isolated from all four inoculated plants as shown in Fig. 5A-B.

Fig. 5. Upper surface of *Rhizoctonia solani* culture re-isolated from original diseased rhizome from the Auburn University Medicinal Garden (A), reverse side of *R. solani* re-isolate (B), and magnified culture, 200x (C).

A.  B.  C. 

The fungus was morphologically identified in each of the samples by septa (characteristic of higher fungi), near-right angle branching, and the absence of conidia and no clamp connections as described by Duggar (1915) as shown in Fig. 5C.

Because rhizome rot of turmeric can contribute to devastating economic losses (Sarathi et al., 2014), an integrated fungal pest management plan should be devised by turmeric growers as *Rhizoctonia solani* is an endemic pest to Alabama soils (Palmateer et al., 2004). Common turmeric production practices in India provide a basic knowledge that can be evaluated and refined for turmeric production in the southeastern U.S. Because the disease is both seed- and soil-borne, clean planting material and a clean seedbed are two of the most determining factors of a successful turmeric crop (Sarma and Anandaraj, 2000). In Indian production, rhizomes are treated with fungicide Mancozeb™ (0.3%) for 30 minutes before storing after harvest and again prior to planting the following season to prevent dispersal of the disease through seed material (Jayashree et al., 2015). Fungicide use in terms of product selection, timing of application, and type of application (i.e. seed treatment versus soil drench) should be established to promote the sanitation of seed material. To create soil antagonistic to rhizome rot, Sarma and Anandaraj (2000) recommended mixing soil and organic amendments such as neem cake or ground nut cake with *Trichoderma* as a biocontrol agent. While plant by-products such as neem cake are not common in the U.S., a host of other organic soil amendments such as fertilizers, mulches, and composted and green manures are available for investigation for soil improvement. As turmeric becomes a more prominent niche crop in Alabama and the Southeast, research needs will become more evident and refined.

Overall Conclusions

Curcuma accessions grown on the campus of Auburn University demonstrate both *Meloidogyne incognita* and fungal susceptibility. Though intraspecific variation in plant growth was observed in terms of *M. incognita* infestation among the *C. longa* accessions, all accessions demonstrated susceptibility to the nematode pathogen within the first 60 days of development.

Curcuma longa 2 and *C. longa* 4 demonstrated increased plant development, decreased nematode population density, or both in microplot and greenhouse screening. Although these accessions are considered low in curcumin, the proliferative growth and exceptional yield of these accessions may provide an edge over slower growing accessions in *M. incognita*-infested areas. Because no nematicides are currently labeled for turmeric in the U.S., alternative means of nematode management will pose great opportunity for future research. In addition to the presence of *M. incognita,* potential turmeric growers should monitor the presence of fungal pathogens. Turmeric serves as a host to endemic fungal pests including *Rhizoctonia solani* and *Macrophomina phaseolina*. A successful means of fungal pathogen management has yet to be determined for Alabama turmeric production. Effective and sustainable means of both nematode and fungal management strategies will provide the crucial next step for turmeric production in Alabama.

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Table 1. List of *Curcuma* spp. accessions present in the Medicinal Plant Garden turmeric plots located on the campus of Auburn University, Auburn, AL.

Curcuma accession	Source	Original geographic source
C. aeruginosa	Lam Duong, Auburn University, Auburn, AL	Vietnam
C. amada	Fort Valley State University, Fort Valley, GA	Unknown
C. caesia 2	Lam Duong, Auburn University, Auburn, AL	Vietnam
C. caesia 3	Lam Duong, Auburn University, Auburn, AL	Vietnam
C. longa 2	International Farmers Market, Atlanta, GA	Unknown
C. longa 3	Horizon Herbs, Oregon	Hawaii
C. longa 4	Le Jardin Ombrage, FL	Unknown
C. longa 5	James Simon, Rutgers University, New Brunswick, NJ	India
C. longa 6	James Simon, Rutgers University, New Brunswick, NJ	India
C. longa 7	James Simon, Rutgers University, New Brunswick, NJ	Korea
C. longa 8	James Simon, Rutgers University, New Brunswick, NJ	South Asia
C. longa 9	Lam Duong, Auburn University, Auburn, AL	Vietnam
C. zeodaria	International Farmers Market, Atlanta, GA	Unknown

Table 2. *Meloidogyne incognita* population density from post-harvest soil samples of *Curcuma longa* accessions adjacent to the Auburn University Medicinal Garden plots, Auburn, AL[y].

Curcuma accession	*M. incognita* J2/150cc of soil
C. longa 2	392 b[z]
C. longa 3	293 b
C. longa 4	264 b
C. longa 5	1284 a
C. longa 6	222 b
C. longa 7	331 b
C. longa 8	141 b
C. longa 9	244 b

[y]Rhizomes were planted in mid-April 2015 and harvested in January-early February 2016. Soil samples were collected shortly after harvest on February 24, 2016.
[z]Means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly.

Table 3. *Curcuma longa* accession response to *Meloidogyne incognita* race 3 measured by plant height, shoot and root fresh weight, and *M. incognita* eggs per gram of root in greenhouse trials, 60 DAP[x].

Curcuma accession	Plant height (PH) (cm)	Shoot fresh weight (SFW) (g)	Root fresh weight (RFW) (g)	*M. incognita* eggs/g root	Reproductive factor (RF)
C. longa 2[y]	50.9 a[z]	23.5 a	37.6 b	564 ab	8.3
C. longa 3	46.2 ab	14.8 bc	27.1 b	701 ab	9.0
C. longa 4	47.7 ab	14.5 bc	30.8 b	196 b	3.9
C. longa 5	41.3 b	12.5 c	27.6 b	558 ab	5.9
C. longa 6	38.1 c	12.3 c	32.5 b	808 ab	8.9
C. longa 7	46.8 ab	18.0 b	52.1 a	281 ab	4.9
C. longa 8	38.1 c	12.9 c	25.4 b	844 ab	10.4
C. longa 9	46.7 ab	18.0 b	30.6 b	1489 a	8.5

[x]Days after planting (DAP), test was repeated once (N=10).
[y]At 30 DAP, accessions (N=10) were inoculated with 2,000 eggs and J2 *M. incognita* race 3.
[z]All values presented are LS-means separated using the Tukey-Kramer method at $P \leq 0.1$. Values in the same column followed by the same letter do not differ significantly.

Table 4. *Curcuma longa* accession response to *Meloidogyne incognita* race 3 measured by plant height, shoot and root fresh weight, *M. incognita* eggs per gram of gram of root, and total *M. incognita* population density in microplot trials, 60 DAP[y].

Curcuma accession	Plant height (PH) (cm)	Shoot fresh weight (SFW) (g)	Root fresh weight (RFW) (g)	*M. incognita* eggs/g root	Total *M. incognita* population density
C. longa 2	53.3 a[z]	114.4 a	77.3 a	301 c	29571 a
C. longa 3	34.2 bc	40.8 ed	17.1 c	1505 a	26288 a
C. longa 4	53.5 a	100.8 b	80.0 a	384 bc	42387 a
C. longa 5	37.2 bc	33.2 e	29.6 c	1434 a	37319 a
C. longa 6	31.8 c	31.9 e	23.9 c	1101 ab	22920 a
C. longa 7	41.5 b	87.5 c	58.4 a	889 abc	29285 a
C. longa 8	31.7 c	40.0 ed	28.2 c	827 abc	24506 a
C. longa 9	39.8 b	50.3 d	31.5 b	997 ab	29077 a

[y]Days after planting (DAP), test was repeated once (N=10).
[z]All values presented are LS-means separated using the Tukey-Kramer method at $P \leq 0.1$. Values in the same column followed by the same letter do not differ significantly.

Table 5. Efficacy of chemical nematicide treatments on *Curcuma longa* 2 measured by plant height, shoot and root fresh weight, *Meloidogyne incognita* eggs per gram of gram of root, and total *M. incognita* population density in field trials, 90 DAP[y].

Treatment, rate	Plant height (PH) (cm)	Dunnett's *P* versus (P≤0.1)	Shoot fresh weight (SFW) (g)	Dunnett's *P* versus (P≤0.1)	Root fresh weight (RFW) (g)	Dunnett's *P* versus (P≤0.1)	*M. incognita* eggs/g root	Dunnett's *P* versus (P≤0.1)	Total *M. incognita* population density	Dunnett's *P* versus (P≤0.1)
Untreated Control	44.3[z]	----	106.3	----	39.1	----	477	----	5015	----
Velum Total (fluopyram+ imidaclopird), 18 fl. oz./A	47.9	0.9990	95.7	1.0000	23.5	0.9394	293	0.9543	3788	0.9921
Counter (terbufos), 6 oz./1000 row feet	37.6	0.9762	62.3	0.9510	26.9	0.9777	140	0.6388	2009	0.7027
Avicta (abamectin), 16 fl. oz./A	55.0	0.8307	183.6	0.6583	51.1	0.9798	269	0.9238	2736	0.8763
Vydate-L (oxamyl), 5 gal./A	58.5	0.6220	162.4	0.8715	51.1	0.9803	34	0.3815	343	0.2984
Movento (spirotetramat), 5 fl. oz./A	45.3	1.0000	114.8	1.0000	32.4	0.9990	453	1.0000	5572	0.9999
Majestene (*Burkholderia* sp., strain A396), 8 qt./A	58.5	0.6220	129.6	0.9979	65.4	0.6378	148	0.6596	1700	0.6195

[y]Days after planting (DAP), test was repeated once (N=8).
[z]All values presented are LS-means. Adjusted means were obtained by analyzing data according to Dunnett's method. Adjusted P-values less than 0.1 indicated a significant effect.

Table 6. List of fungal genera (and species where applicable) extracted from the turmeric plots at the Auburn University Medicinal Garden.

Sample No.	Host *Curcuma* sp.	BLAST description	Max Score[u]	Total Score[v]	Query Cover[w]	E Value[x]	Ident[y]	Accession[z]
2	*C. caesia* 3	*Fusarium oxysporum*	453	907	91%	2.00E-123	100%	KT794176.1
6	*C. longa* 9	*Fusarium* sp.	1037	1819	97%	0	93%	JQ364977.1
		Fusarium solani	941	1599	97%	0	100%	KR528470.1
10	*C. zeodaria*	*Fusarium* sp.	793	793	100%	0	100%	KM655521.1
14	*C. longa* 2	*Fusarium oxysporum*	785	785	100%	0	100%	KT898677.1
		Fusarium sp.	785	785	100%	0	100%	KU831532.1
18	*C. longa* 9	*Fusarium oxysporum*	789	789	100%	0	100%	KT898677.1
		Fusarium sp.	789	789	100%	0	100%	KU831532.1
22	*C. caesia* 2	*Fusarium oxysporum*	785	785	100%	0	100%	KT898677.1
		Fusarium sp.	785	785	100%	0	100%	KU831532.1
24	*C. caesia* 2	*Fusarium verticillioides*	793	793	100%	0	100%	KU527425.1
25	*C. longa* 7	*Fusarium oxysporum*	791	791	100%	0	100%	KT898677.1
		Fusarium sp.	791	791	100%	0	100%	KU831532.1
26	*C. amada*	*Fusarium oxysporum*	808	808	100%	0	99%	KT877649.1
		Fusarium proliferatum	808	808	100%	0	99%	KU687107.1
29	*C. caesia* 3	*Fusarium oxysporum*	817	817	100%	0	100%	KT877649.1
		Fusarium proliferatum	817	817	100%	0	100%	KU687107.1
30	*C. caesia* 2	*Fusarium oxysporum*	817	817	100%	0	100%	KT877649.1
		Fusarium proliferatum	817	817	100%	0	100%	KU687107.1
32	*C. aeruginosa*	*Fusarium oxysporum*	813	813	100%	0	100%	KT877649.1
		Fusarium proliferatum	813	813	100%	0	100%	KU687107.1
33	*C. aeruginosa*	*Fusarium oxysporum*	791	791	100%	0	100%	KT898677.1
		Fusarium sp.	791	791	100%	0	100%	KU831532.1
34	*C. longa* 2	*Fusarium oxysporum*	791	791	100%	0	100%	KT898677.1
		Fusarium sp.	791	791	100%	0	100%	KU831532.1

36	*C. longa* 4	*Fusarium oxysporum*	787	787	100%	0	100%	KT455376.1
37	*C. longa* 2	*Fusarium oxysporum*	793	793	100%	0	100%	KT455376.1
38	*C. longa* 2	*Fusarium verticillioides*	795	795	100%	0	100%	KU527425.1
42	*C. longa* 4	*Fusarium oxysporum*	595	595	94%	2.00E-166	100%	KT794176.1
43	*C. longa* 4	*Fusarium sacchari*	610	610	94%	6.00E-171	100%	KR071659.1
44	*C. longa* 3	*Fusarium* sp.	793	793	95%	0	100%	KM655521.1
		Fusarium fujikuroi	793	793	95%	0	100%	KJ000438.1
		Fusarium sacchari	793	793	95%	0	100%	KC464631.1
45	*C. longa* 2	*Fusarium subglutinans*	785	785	95%	0	99%	KP773287.1
		Fusarium proliferatum	785	785	95%	0	99%	KP760063.1
		Fusarium verticilliodes	785	785	95%	0	99%	KP003945.1
46	*C. longa* 9	*Fusarium oxysporum*	915	915	96%	0	100%	KR856356.1
47	*C. amada*	*Fusarium oxysporum*	813	813	95%	0	100%	KT794176.1
48	*C. amada*	*Fusarium oxysporum*	791	791	95%	0	100%	KT794176.1
49	*C. amada*	*Fusarium oxysporum*	817	817	100%	0	100%	KT877649.1
		Fusarium proliferatum	817	817	100%	0	100%	KU687107.1
3	*C. longa* 7	*Ceratobasidium* sp.	1038	2077	96%	0	100%	KJ573103.1
		Rhizoctonia sp.	1038	2077	96%	0	100%	JF519837.1
9	*C. aeruginosa*	*Ceratobasidium* sp.	1064	1064	100%	0	100%	KJ573103.1
		Rhizoctonia sp.	1064	1064	100%	0	100%	JF519837.1
13	*C. amada*	*Ceratobasidium* sp.	1040	1040	100%	0	100%	KJ573103.1
		Rhizoctonia sp.	1040	1040	100%	0	100%	JF519837.1
17	*C. caesia* 2	*Ceratobasidium* sp.	1053	1053	100%	0	100%	KJ573103.1
		Rhizoctonia sp.	1053	1053	100%	0	100%	JF519837.1
21	*C. caesia* 2	*Ceratobasidium* sp.	1050	1050	100%	0	100%	KJ573103.1
		Rhizoctonia sp.	1050	1050	100%	0	100%	JF519837.1
28	*C. longa* 9	*Ceratobasidium* sp	1013	1013	100%	0	100%	KJ573103.1
		Rhizoctonia sp.	1013	1013	100%	0	100%	JF519837.1

40	*C. longa* 2	*Ceratobasidium* sp.	1038	2077	98%	0	100%	KJ573103.1
		Rhizoctonia sp.	1038	2077	98%	0	100%	JF519837.1
51	*C. amada*	*Ceratobasidium* sp.	1046	2092	98%	0	100%	KJ573103.1
		Rhizoctonia sp.	1046	2092	98%	0	100%	JF519837.1
55	*C. amada*	*Ceratobasidium* sp.	1029	2059	98%	0	100%	KJ573103.1
		Rhizoctonia sp.	1029	2059	98%	0	100%	JF519837.1
7	*C. longa* 2	*Trichoderma virens*	941	941	96%	0	100%	KT278905.1
8	*C. caesia* 2	*Trichoderma gamsii*	924	924	96%	0	100%	KT037690.1
12	*C. caesia* 2	*Trichoderma harzianum*	963	963	100%	0	100%	KU319056.1
		Trichoderma sp.	963	963	100%	0	100%	KT264647.1
16	*C. longa* 7	*Trichoderma harzianum*	941	941	100%	0	100%	KU145463.1
		Trichoderma afroharzianum	941	941	100%	0	100%	KT278892.1
4	*C. zeodaria*	*Diplodia seriata*	889	1778	95%	0	99%	FJ790841.1
1	*C. zeodaria*	*Macrophomina phaseolina*	880	880	95%	0	100%	FJ395243.1
5	*C. longa* 3	*Nigrospora* sp.	392	392	91%	1.00E-105	100%	HQ631070.1
11	*C. longa* 9	Unidentifiable						
15	*C. longa* 4	Unidentifiable						
19	*C. longa* 7	Unidentifiable						
20	*C. longa* 7	Unidentifiable						
23	*C. amada*	Unidentifiable						
27	*C. longa* 6	Unidentifiable						
31	*C. zeodaria*	Unidentifiable						
35	*C. caesia* 2	Unidentifiable						
39	*C. longa* 2	Unidentifiable						
41	*C. caesia* 3	Unidentifiable						
50	*C. amada*	Unidentifiable						
52	*C. amada*	Unidentifiable						
53	*C. amada*	Unidentifiable						

54	*C. amada*	Unidentifiable						

[u]Score of single best aligned sequence.
[v]Sum of scores of all aligned sequences.
[w]Percentage of query sequence aligned.
[x]Number of matches with same score expected by chance, or the probability of random alignment. Typically, E<0.05 is considered significant.
[y]Extent to which two sequences have the same residues in alignment, expressed as a percentage.
[z]Distinguishing identifier of a sequence record that allows for tracking of different versions of the sequence.

Table 7. Number of isolates and mean isolation frequency (MIF) of fungal genera collected from diseased rhizomes in the turmeric plots located at the Auburn University Medicinal Garden, Auburn, AL, where sample size = 41.

Fungus	Number of isolates	MIF[z]
Fusarium sp.	25	60.9
Rhizoctonia solani	9	21.9
Trichoderma sp.	4	9.7
Macrophomina phaseolina	1	2.5
Nigrospora sp.	1	2.5
Diplodia seriata	1	2.5

[z]MIF=[Number of isolates of a genus/Total number of samples]*100