2013

The influence of Commerce silt loam soil texture on reproduction and pathogenicity of Rotylenchulus reniformis on cotton

Déborah Magalhães Xavier
Louisiana State University and Agricultural and Mechanical College

Recommended Citation

THE INFLUENCE OF COMMERCE SILT LOAM SOIL TEXTURE ON REPRODUCTION
AND PATHOGENICITY OF *ROTYLENCHULUS RENIFORMIS* ON COTTON

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Plant Pathology and Crop Physiology

by
Déborah Magalhães Xavier
B. S., Federal University of Viçosa, 2009
May 2013

To God who made all things possible.

To my parents and my sister, for their never ending love and prayers.

To the memory of my grandpa. Vô Jacy, I will always remember you!

Avellaneda, the Brazilian community in Baton Rouge, and all my friends in here and in Brazil for sharing tears and laughs, and for giving balance to my life.

I am also grateful to my beloved Kevin, for always having a smile in his face and a word of encouragement for me. Thanks for being my happy company, and for taking care of me when I didn't have time to do it myself. Thank you for never letting me lose faith in myself.

I am thankful to the students, faculty and staff of the Department of Plant Pathology and Crop Physiology at LSU. Thank you for the warm welcome and for your help during these years. Special thanks to my English tutors: Nicole Ward and Rebecca Sweany. I could not have proceeded without those first steps.

I could not have completed this journey without the support and prayers from my family members, especially my parents, Marisa Magalhães Xavier and Manoel Joaquim Xavier Filho, and my sister, Mírian Magalhães Xavier. I will be forever grateful for everything you have done for me. I thank my aunts, grandmother and cousins for their support and for understanding my absence.

Last but not least, I would like to acknowledge my aunt Maria Auxiliadora Xavier (tia Dorinha), and my uncles José Carlos Xavier (tio Cacá) and José Magalhães (tio Zé) that passed away during the course of this work. Also in memory of my lovely aunts Maria de Oliveira Xavier (tia Zizi) and Leila Xavier (tia Leila), for their inspiring faith and contagious happiness. Unfortunately they will not able to see me with this degree in life. Thanks for interceding for me with God! Somehow you were also part of this achievement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Greenhouse and microplot studies were conducted to evaluate the influence of soil texture on reproduction and pathogenicity of *Rotylenchulus reniformis* (reniform nematode) on cotton. A 45 day duration greenhouse experiment confirmed the pathogenicity of an isolate of *R. reniformis* from Avoyelles Parish on Stoneville LA887 cotton. A series of greenhouse experiments were conducted with three geographic isolates of *R. reniformis* (identified as Avoyelles, Evangeline, and Rapides to indicate the Parish of origin) on Stoneville LA887, Stoneville 5288B2F, and Phytogen 375WF cotton growing in soils with varying textures for 60 days. Soil types with sand, silt, and clay contents ranging from 74.4 to 7.8, 20.7 to 66.3, and 4.9 to 25.9, respectively, were employed in this research. Two experiments were conducted with Stoneville LA887 cotton, three soil types and Avoyelles isolate of reniform nematode for 150-152 days in a microplot environment. In the greenhouse, variations in soil texture significantly affected plant height and dry weights in both Stoneville 5288B2F and Phytogen 375WF, but did not have any significant effect on plant growth of Stoneville LA887, except in the 45-day duration experiment. Stoneville 5288B2F plants were significantly taller throughout the experiment in soil with 31.4% sand and 13.3% clay. Phytogen 375WF cultivar showed the same pattern, but the difference in plant height was not observed at harvest. Stoneville 5288B2F and Phytogen 375WF had significantly reduced dry root and shoot weights in sandier soils. Soil type had a significant effect on nematode reproduction on all three cotton cultivars. The interaction between soil type and reniform isolate significantly affected population densities of all reniform isolates tested among the multiple soil types in the cultivars Stoneville 5288B2F and Phytogen 375WF, but no effect was observed in Stoneville LA887 cultivar. In the microplot, plants growing in soil with more clay content had significantly greater root and shoot weights than the

others. The number of bolls open and seed cotton weight were significantly reduced by *R. reniformis* in the microplots. Population densities of *R. reniformis* in the microplot followed the same pattern observed in greenhouse experiments.

INTRODUCTION

Cotton is grown in 17 states in the United States and had a 3.7 billion dollar impact on the economy in 2009 with more than 3 million hectares harvested (Anonymous, 2010b). Production in Louisiana represented about 3% of this total (Anonymous, 2010a). Despite the 15% reduction in area planted from 2011 to 2012, there was an increase of about 9% in yield over these years (Anonymous, 2013). Last year, Louisiana cotton yield ranked 8th out of the 17 cotton growing states in the U.S. with an average yield of 1,124 kg per hectare.

According to the National Cotton Council of America (2010), cotton yield losses due to nematodes have increased substantially since 1990. Several nematode species have been reported as a potential threat to cotton in the United States (Smith and Taylor, 1941; Robinson et al., 1987; Koenning et al., 2004; Gazaway, 2005). Root-knot nematode (*Meloidogyne incognita*) has historically been the most damaging nematode of cotton. In recent years, reniform nematode (*Rotylenchulus reniformis*) has replaced root-knot nematode as the most damaging species in the Southeastern states of the U.S. (Gazaway, 2005; Overstreet and McGawley, 1998; Robinson, 2007). This shift in nematode dominance may be associated with the higher rate of survival over winter demonstrated by reniform populations (Koenning et al., 1996; Robinson, 2007). Koenning et al. (1996) further suggested that the high levels of reniform nematode in cotton fields in North Carolina reflect the ability of this nematode to establish feeding sites all along the root system, whereas root-knot nematode establishes feeding sites primarily at the root tip. Variation in reproduction and pathogenicity within and among geographical isolates *R. reniformis* has been confirmed both in soybean (McGawley et al., 2011) and cotton (Agudelo et al., 2005; McGawley et al., 2010). Such variation in reniform reproduction and pathogenicity is likely to be a significant factor impacting the search for new resistance germplasms (McGawley et al., 2012).

1

Currently, *R. reniformis* is one of the most important pests of cotton in Louisiana and other southern states with yield losses estimated at $166 million (U.S. dollars) in 2004 (Robinson, 2007; Koenning et al., 2004; Gazaway, 2005). According to the National Cotton Council, losses due to reniform nematode in cotton have increased steadily over the past years throughout the cotton growing areas of the United States. Yield loss due to *R. reniformis* in the Beltwide Cotton areas of the U.S. in 2011 was approximately 280,000 bales (Blasingame and Patel, 2012). In Louisiana, reniform nematode was responsible for the loss of about 23,000 bales in 2011 (Blasingame and Patel, 2012).

Reniform nematode was first described by Linford and Oliveira in Hawaii in 1940. In Louisiana, it was identified as a pest in 1941 by Smith and Taylor on cotton and cowpea and it was associated with stunted cotton in 1960s (Birchfield and Jones, 1961). At that time, fields with high levels of infestation showed poor stands (Birchfield, 1962) and growers began to be concerned about the importance of controlling reniform nematode. Since then, the occurrence and losses due to this nematode have increased throughout the state of Louisiana (Overstreet and McGawley, 1996; 2000).

Reniform nematode causes reductions in yield, delays in maturity, and reductions in boll size and lint percentage (Birchfield and Jones, 1961; Overstreet and Wolcott, 2007). According to Blasingame et al. (2008), approximately 4% of cotton losses in Louisiana in 2007 were the result of *R. reniformis*.

Although some progress has been made towards producing upland cotton with resistance to reniform nematode, currently there is no commercial resistance. The search for resistance remains a subject of intense research (Robinson and Percival, 1997; Koenning et al., 2004; Weaver, et al., 2007; Agudelo, P., 2007; Romano et al., 2009; Parkhi, et al., 2010). Although

possessing some resistance to reniform nematode, the LONREN germplasm, released in 2007, has been reported to exhibit stunting and low yield in fields with high levels of reniform nematode (Nichols et al., 2010). In 2009, Romano et al. identified molecular markers related to a resistance source that might be useful in developing cotton varieties with resistance to *R. reniformis*. LONREN and BARBREN germplasm lines with potential resistance to reniform nematode have performed poorly in the field when compared with commercial cultivars (Sikkens et al., 2012).

Aside from genetic resistance, other possible management strategies for *R. reniformis* in cotton are crop rotation, biological control, and nematicide application. Since cotton is such a good host for the reniform nematode, management strategies frequently require a combination of these approaches to be efficient (Blasingame et al., 2008).

As related by Cabanillas et al. (1999), crop sequence influenced population densities of *R. reniformis*. In a crop rotation study, plots where cotton was followed by corn had greater population densities of reniform nematode than plots where fallow was used after corn, grain sorghum, or cotton. Despite reducing nematode levels, adopting fallow as a management practice is not always economically viable. Using non-host or poor host crops may also affect nematode densities, since the nematode populations will not increase as rapidly in these crops as on cotton (Stetina et al., 2007). The advantage of crop rotation for managing reniform nematode is limited in many areas by both the lack of resistant crops that will provide economic return and by the ability of populations to increase rapidly when cotton is introduced into the area (Barker and Koenning, 1998; Davis et al., 2003, Stetina et al., 2007). Another issue to be considered is that the presence of multiple nematode species in the same area makes it difficult to find a crop that is not a host for all indigenous nematode species. *R. reniformis* and *M. incognita* are increasingly

being reported as cohabiting cotton growing areas in the mid-South and Southeast United States (Overstreet et al., 2010).

Some biological control agents have been shown to be efficacious against the reniform nematode. Most of these agents are nematophagous fungi. Walters and Barker (1994) demonstrated the efficiency of *Paecilomyces lilacinus* in suppressing *R. reniformis* population on tomato under both greenhouse and microplot conditions. Wang et al. (2005) demonstrated that *Pochonia chlamydosporia* isolates from fields in Arkansas were able to parasitize eggs of *R. reniformis* and to decrease their population density in greenhouse conditions. The bacterium *Pasteuria* spp. showed efficiency in parasitizing *R. reniformis* in vitro (Hewlett et al., 2010). It is likely that ideal management will be accomplished by combining biological control with other management techniques.

Traditionally, nematicides have been the most common management strategy for nematodes (Koenning et al., 2004; Starr et al., 2007; Overstreet et al., 2007). In cotton, aldicarb has been the most widely used nematicide in the U.S. (Koenning et al., 2004). Production of aldicarb ceased in the United States in 2011 (C. Overstreet, pers. comm.). The use of nematicides as a short term management strategy has resulted in increased yield and plant vigor (Davis et al., 2003; Faske and Starr, 2005; McGawley et al., 2006; Overstreet et al., 2007), but it has also increased the cost of production and elicited environmental concern (Starr et al., 2007). Avicta Complete Cotton (Syngenta) and AERIS Seed-Applied System (Bayer CropScience) are currently the dominant seed treatment nematicides used with cotton in the U.S. (Erwin et al., 2010). To make the use of seed treatment nematicides economically viable, it will probably be necessary to integrate their use with other management practices, such as crop rotation. In a two

4

year rotation of cotton with peanut and corn combined with aldicarb application, reniform nematode population density was reduced by 86% (Royal and Hammes, 2005).

Another potential nematode management strategy involves the use of precision technology. Dividing a field into zones according to soil texture, nematode species, and/or yield will make possible the establishment of management zones within the field (Barker and Koenning, 1998; Wyse-Pester, et al., 2002; Koenning et al., 2004, Erwin et al., 2007). Such parameters can enable the creation of site specific management (SSM) zones. Evans et al. (2003) based the application of a nematicide on nematode populations for specific areas within the field. SSM will reduce nematicide costs, since they will be applied only in sites where there is a yield response to its application (Starr et al., 2007; Erwin et al., 2007). The SSM system has also been successfully applied to delineate management zones associated with *Meloidogyne incognita* on cotton (Perry, C. et al., 2006; Ortiz et al., 2007, 2012) and *Heterodera glycines* on soybean (Avendaño et al., 2004a, 2004b, 2004c).

ROLE OF SOIL TEXTURE IN NEMATODE MANAGEMENT

Soil texture is an important characteristic to be considered when managing nematodes affecting agronomic crops (Sivakumar and Seshadri, 1972; Robinson et al., 1987; Koenning et al., 1996; Herring et al., 2010). Soil texture will affect nematode management because it affects their damage potential (Barker and Weeks, 1981; Griffin, 1996; Overstreet et al., 2010, 2011a).

In 1988, Koenning et al. demonstrated that damage caused on susceptible cultivars of soybean by the soybean cyst nematode (SCN), *Heterodera glycines*, was strongly related to soil texture. Similarly, Avendaño et al. (2004b) found that soils with sand contents greater than 60% were able to withstand greater populations of SCN, confirming the relationship between SCN spatial distribution and soil texture. The greater the sand content in the soil, the more likely soybean plants are to be severely damaged by SCN (Koenning et al., 1988; Avendaño et al., 2004a, 2004b, 2004c). Some studies have demonstrated a correlation between the root-knot nematode, *M. incognita*, and soil texture in cotton and/or in soybean fields (Koenning et al., 1996; Shane and Barker, 1986; Ortiz et al., 2007; Monfort et al., 2007). This research demonstrated that greater population densities of the root-knot nematode are associated with coarse textured soils. Shane and Barker (1986) also found that soybean growth was significantly influenced by nematodes in soils with greater sand contents. Similar observations were made by Koenning et al. (1996) while studying soil texture influences on the reproduction and damage potential of both *M. incognita* and *R. reniformis* on cotton. The use of SSM for root-knot nematode on cotton based on readings of apparent electrical conductivity (EC_a) has been successfully applied in cotton fields in Arkansas, affirming the potential of using this strategy for predicting areas most likely to be damaged by this nematode (Monfort et al., 2007).

Reproduction of *R. reniformis* also has been shown to be significantly influenced by soil texture. Several studies demonstrated that this nematode, unlike root-knot, is favored by finer textured soils (Robinson et al., 1987; Sivakumar and Seshadri, 1972; Koenning et al., 1996; Overstreet et al., 2010; Moore and Lawrence, 2011; Overstreet et al., 2011a). Zhao et al. (2000) found that in silt loam and loamy sand soils the population density of reniform nematode was greater on soybean than on cotton. However, on finer textured soils, there was no difference in population density between the two crops. In Louisiana, most cotton fields are located in areas where the predominant soil type is a silt loam, in which reniform nematode can reproduce rapidly and cause significant yield losses. Commerce silt loam soil is a common soil type found in cotton production areas in Northeast Louisiana. It is exactly this soil type in which the most variable response to nematicide application has been observed (Overstreet et al., 2011b).

In this context, precision farming techniques are extremely important to maximize crop profitability. Improvements in the geographical information system (GIS) associated with the global position system (GPS) have been essential in formulating management decisions in current agriculture (Thomas et al., 2002; Ortiz et al., 2012; Monfort et al., 2007). The adoption of SSM zones will increase the accuracy and precision required for successful and profitable nematode management. However, in order to successfully use the SSM technology, new variables such as the spatial distribution of the nematodes and the effect of soil texture on nematode damage potential will need to be characterized (Avendaño et al., 2004a, b, c; Koenning et al., 1996; Melakeberhan, 2002). One feasible alternative for accessing soil texture is the application of apparent electrical conductivity (EC_a) technology (Ortiz et al., 2007, 2012; Xavier, et al., 2012). As affirmed by Ortiz et al. (2012), the correlation between aggregated spatial distribution of *M. incognita* and soil texture makes SSM a feasible management strategy for this

nematode. Grid sampling or zone sampling for nematodes can be combined with EC_a data to map nematode populations within a field.

It is essential to embrace current technologies in order to develop twenty first century integrated nematode management techniques. With *R. reniformis* in particular, the refinement of conventional techniques is essential because they have been ineffective against this pathogen, especially on cotton. This research is focused on the influence of soil texture (Commerce silt loam soil in particular, since it is a major type associated with cotton production in Louisiana) on the reproduction and pathogenicity of the reniform nematode, *R. reniformis*.

MATERIALS AND METHODS

Isolates of Reniform Nematode

Isolates of reniform nematode from Louisiana were supplied/collected by E. C. McGawley. Three geographic isolates (identified as Avoyelles, Evangeline, and Rapides to indicate the Parishes where they were originally collected) were obtained from axenic cultures maintained in the LSU Nematology greenhouse on tomato (cultivar Rutgers PS Seedway; Hall, New York 14463) for use as inoculum. Each of these three isolates has been confirmed *R. reniformis* as described by McGawley et al, 2010 and Robinson et al, 1997.

General Information

In the greenhouse, terra cotta pots with an inside top diameter of 10.2 cm or 15.0 cm holding 0.5 or 1.6 kg of soil, respectively, were used. Microplots were terra cotta pots having an inside top diameter of 42.9 cm with a soil capacity of 27.3 kg. In all experiments, all materials including soils were autoclaved prior to use. All experiments were repeated once.

The soil used in the greenhouse and microplot studies originated from the Northeast Research Station, located at St. Joseph, Louisiana. Soil from three different sites within the same field (Appendix, Figures A1 and A2) was selected based on apparent electrical conductivity (EC_a) values obtained employing a Veris® Soil EC Mapping System. The EC_a data was measured from two soil depths: 0-0.3 m or shallow EC_a (EC_{a-sh}), and 0-0.9 m or deep EC_a (EC_{a-dp}). The EC_a data was processed utilizing SSToolbox (Anonymous, 2011b). The EC_{a-sh} and EC_{a-dp} data points were interpolated to a 6.1 m x 6.1 m grid cell format using the Kriging tool of SSToolbox and classified into zones using unsupervised natural breaks. Figures A1 and A2 (Appendix) show the field divided into 10 zones based on the EC_{a-sh} and EC_{a-dp} values, respectively. Figure A3 (Appendix) shows that all the samples were collected from the same soil

zone (Commerce silt loam, fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts). The location of each site was georeferenced in the field using a Trimble Juno handheld GPS receiver and a FarmWorks SiteMate Pro program. Soil from the three sites differed in sand, silt and clay content according to the Hydrometer method modified from Day (1965) and the American Society for Testing and Materials (1985) (Table 1).

Table 1. Soil texture analysis and apparent electrical conductivity (EC_a) from three sites within a Commerce silt loam field on the Northeast Research Station in St. Joseph, LA used in greenhouse and microplot studies.

Soil type	Particle size distribution (%)			EC_a (mS/m)[X]	
	Sand	Silt	Clay	EC_{a-sh}	EC_{a-dp}
T1	74.4	20.7	4.9	7.4	27.5
T2	31.4	55.3	13.3	8.7	49.9
T3	7.8	66.3	25.9	33.4	88.1

[X]Apparent electrical conductivity (EC_a) data was measured in millisiemens per meter (mS/m) from two soil depths: 0-0.3 m or shallow EC_a (EC_{a-sh}), and 0-0.9 m or deep EC_a (EC_{a-dp}).

Additionally, soil samples from each site were sent to the LSU Soil Testing and Plant Analysis Lab for nutrient analysis (Table 2). According to recommendations from the LSU Soil Testing Lab, the pH of soil from sampling site T1 was low (pH of 5.4). Soil from this site was amended with calcium hydroxide at a rate of 1,120 kg per hectare, or 13.7 g or 0.8 g per microplot or pot respectively, to adjust pH to 7.0. The pH amendment was performed after planting in greenhouse experiment 2 and prior to planting in greenhouse experiments 3 and 4, and in microplots.

Table 2. Nutrient status of soil from three sites within a Commerce silt loam field at the Northeast Research Station in St. Joseph, LA[X].

Soil type	Ca[Y]	Cu[Y]	Mg[Y]	pH	P[Y]	K[Y]	Na[Y]	S[Y]	Zn[Y]	OM[z]
T1	692.3	0.4	146.5	5.4	53.5	129.5	5.8	12.9	1.6	0.7
T2	1494.0	1.5	173.0	7.2	47.6	161.1	7.4	13.7	3.1	1.1
T3	2520.2	2.4	483.5	6.8	60.4	270.8	3.3	18.2	3.7	1.7

[X]Values are averaged over two replications.
[Y]Values are expressed as mg/kg.
[z]OM indicates percentage of organic matter.

Nematode inoculum for all tests consisted of juveniles, preadult females, and males extracted from greenhouse cultures by wet-sieving through nested 250-µm-pore and 38-µm-pore sieves followed by sugar flotation and centrifugation (Jenkins, 1964). Soils in pots and microplots were infested with nematodes by pipetting aqueous suspensions containing vermiform individuals of *R. reniformis* into a series of depressions arranged into a triangular pattern in soil, 0.5 cm diameter X 5-7.5 cm deep, surrounding the seedling. In the greenhouse studies, three cotton cultivars (Stoneville LA887, Stoneville 5288B2F, and Phytogen 375WF) well known by their field performance (Anonymous, 2011a) and susceptibility to reniform nematodes were used (McGawley et al., 2010; Sikkens et al., 2012). In the microplot, the cultivar Stoneville LA887 was utilized.

In both greenhouse and microplot studies, two cotton seeds were planted in each pot to a depth of 2.5 cm. To optimize seedling establishment, pots were placed inside of or covered by a plastic bag. Bags were removed once seeds were established and thinned to one per pot or microplot. During the course of all experiments, plants were fertilized every two weeks with water-soluble Miracle-Gro fertilizer, containing 18% nitrogen, 18% available phosphate and 21% soluble potash.

Plant height was measured every two weeks in greenhouse experiments 2, 3, and 4, and in microplot studies. Air temperature was measured daily during all greenhouse and microplot experiments. In microplots, soil temperature for the three different soil types was also monitored. Tensiometers were employed to determine when it was necessary to water pots or microplots since there were different soil types. Pots/microplots in all experiments were arranged as a randomized complete block design.

At the conclusion of all experiments, soil samples were processed by semiautomatic elutriation (Byrd et al., 1976) and centrifugal-flotation (Jenkins, 1964). Immature stages of reniform were enumerated at 40X using an inverted microscope. Total population density per pot (Pf) and reproductive value (R, where R = Pf/Pi, Pf is the final population density, and Pi is the initial infestation level) were determined. Eggs of reniform nematode were extracted from fresh root tissue by stirring in 0.6% of NaOCl for 10 minutes (Hussey and Barker, 1973). In greenhouse studies the entire root system was used for egg extraction and in microplot studies a 5 g subsample (randomly selected) was used.

The duration of microplot studies was full season (150-152 days), and the duration of greenhouse studies were 45 days for experiment one and 60 days for experiments 2, 3, and 4. At the conclusion of all experiments, plant height and dry root and shoot weights were determined. Plant shoots were excised and placed into a paper bag prior to drying at 45ºC for two days for the greenhouse experiments and for seven days for the microplot experiments. After egg extraction root material was handled in a similar manner. At the conclusion of all experiments, tissue samples representative of each treatment were collected and submitted to the LSU Soil Testing and Plant Analysis Lab for nutrient analysis.

Greenhouse Studies

A total of eight experiments were conducted in 2011 and 2012 to evaluate reniform nematode pathogenicity and reproduction on cotton. The first two experiments evaluated the influence of soil type on nematode reproduction and pathogenicity, and employed a single isolate of the nematode and a single cultivar of cotton. The remaining six experiments had the same objective, but employed multiple isolates of the nematode, multiple cultivars of cotton, and multiple soil types.

Experiment 1:

This experiment was initiated to evaluate pathogenicity of a single isolate of reniform nematode on Stoneville LA887 cotton growing in either greenhouse soil (72.1% sand, 25.4% silt, and 2.5% clay) or field soil (31.4% sand, 55.3% silt, 13.3% clay). Treatments in this experiment consisted of two soil types (soil from field sampling site T2 and greenhouse soil) and two nematode infestation levels (0 and 5,000 juveniles, preadult females and males per pot). Pots used for this experiment were 10.2 cm in diameter and nematode inoculated pots received 2,500 vermiform individuals at 10 and 30 days after planting. Treatments were replicated five times.

Experiments 2, 3 and 4:

Experiments 2, 3, and 4 each involved nematode isolates, from Avoyelles, Evangeline, and Rapides Parishes, infestation levels of 0 and 10,000 nematodes per pot and three soil types. Each treatment was repeated five to six times. In experiments 2, 3 and 4, respectively, the cotton cultivars Stoneville LA887, Stoneville 5288B2F, and Phytogen 375WF were employed. Additionally, the Commerce silt loam soils for experiments 2, 3 and 4 had sand, silt and clay content percentages of 74.4, 20.7, 4.9; 31.4, 55.3, 13.3; and 7.8, 66.3, 25.9, respectively (Table 1).

Microplot Studies

The microplot setup more closely simulates a field environment. Plants were grown full season, reached full size, and provided an evaluation of nematode impact on yield. Each microplot was established in preformed depressions in soil with only the rim of the pot exposed to maintain constant soil temperature. Microplots were spaced 1-meter apart and arranged as a randomized block design in a six-by-six pattern. The microplot area was bounded by a 17-meter-long by 9-meter-wide aluminum Quonset hut skeletal frame open at both ends and covered with

one layer of clear, 6-millimeter thick polyethylene greenhouse film and one layer of 20% reflective foil cloth (McGawley et al., 2010). The watering was done manually and was based on tensiometer readings for the three soil types.

The microplot study involved the Avoyelles Parish reniform nematode population at infestation levels of 0 and 30,000 individuals per microplot and three soil types, thus providing six treatment combinations replicated each three times.

Once approximately 60% of the bolls were opened, tribufos (Folex) and ethephon (Prep) were applied twice at weekly interval at the rates of 0.6 and 1.5 l/ha, respectively, to induce boll opening and defoliation.

Numbers of bolls and seed cotton weights were determined at the conclusion of each microplot trial. Six fully developed leaves were collected from the midpoint of each plant and used to calculate the leaf area using Assess 2.0 Image Analysis Software (APS Press, St. Paul, MN). Additionally, internode lengths were determined at harvest for each plant.

Data Analysis

Data from greenhouse experiments were examined by analysis of variance (ANOVA) for a 2 x 2 factorial design (soil type x nematode) or 2 x 3 x 3 (nematode x soil type x reniform isolate) using the "Fit Model" module of SAS JMP, version 10.0 (SAS Institute, Cary, NC). Data from the microplot study was a 2 x 3 factorial design (nematode x soil type). Means of data were separated by Student's t-test ($P \leq 0.05$) in greenhouse experiment 1, by Tukey's HSD at $P \leq 0.05$ in greenhouse experiments 2, 3 and 4, and by least significant difference (LSD) at $P \leq 0.05$ in microplot studies.

RESULTS

For each trial, foliar tissue nutrient analyses were compared, and no differences in nutrient levels between treatments were observed (data not shown).

Greenhouse - Experiment 1:

Soil type had a significant effect on plant height, but did not have any significant effect on either root or shoot dry weights (Table 3). The average height of plants growing on field soil was 18.4 cm, and was significantly greater than that of those growing on greenhouse soil, which averaged 16.0 cm. The presence of reniform nematode did not significantly affect any of the plant data collected in this study. Additionally, soil type did not significantly influence nematode reproduction on this study (Appendix, Table A1). Nematode reproductive values for greenhouse and field soil were, respectively, 1.8 and 2.0 in this experiment. Low reproductive values observed in this experiment were likely the result of short experimental duration and winter temperatures during the months when the experiment was conducted.

Table 3. Main and interaction effects (P values) of soil type and nematode on Stoneville LA887 cotton in a greenhouse environment^X.

Source	DF	Plant height	Root weight^Z	Shoot weight^Z
Soil type^Y (S)	1	0.05*	0.12	0.12
Nematode (N)	1	0.83	0.74	0.77
S x N	1	0.14	0.49	0.14

^{X}Data combined over two 45 day duration experiments with five replications each. Data was analyzed with ANOVA and Student's t-test (P ≤ 0.05).
^{Y}Two soil lots were used in this experiment. Percentages of sand, silt, and clay were 72.1, 25.4, and 2.5for the greenhouse lot, and 31.4, 55.3, and 13.3 for the field lot.
^{Z}Data are dry weight obtained after two days at 45ºC.
*Indicates a significant P value.

Greenhouse - Experiment 2:

Main and interactive effects of soil type and reniform isolate, as well as nematode and egg counts, and reproductive values on Stoneville LA887 cotton are summarized in tables 4 and 5, respectively.

Numbers of vermiform stages in the soil and numbers of eggs per gram of root were both significantly influenced by soil type and reniform nematode isolate (Table 4).

Table 4. Main and interaction effects (P values) of soil type and reniform isolate on Stoneville LA887 cotton in a greenhouse environment – Nematode density[X].

Source	DF	Vermiform stages /500 cm^3 of soil	Eggs/g of root
Soil type[Y] (S)	2	< 0.01*	< 0.01*
Reniform isolate[Z] (I)	2	< 0.01*	0.02*
S x I	4	0.21	0.09

[X]Data combined over two 60 day duration experiments with a total of 11 replications. Data was analyzed with ANOVA and Tukey's HSD (P ≤ 0.05).
[Y]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[Z]Reniform nematode isolates were collected from Avoyelles, Evangeline and Rapides Parishes.
*Indicates a significant P value.

Across the three soil types, the least number of nematodes and eggs was found in T3 soil where the clay content averaged 26% (Table 5). The lowest numbers of vermiform stages in soil, eggs per gram of root system, and corresponding reproductive values were those from the Evangeline isolate (Table 5).

Among the three soil types, final population densities for the Avoyelles isolate ranged from 154,764 to 387,631 vermiform stages per pot with resultant reproductive values from 15.5 to 38.8. Similarly, final population densities for the Evangeline isolate ranged from 117,202 to 219,788 with R values of 11.7 to 22.0. Those for the Rapides isolate ranged from 234,775 to 436,038 with R values averaging from 23.5 to 43.6 (Table 5).

The interaction of soil type and reniform isolate approached significance, especially with respect to eggs per gram of root. This data is presented as Figure 1. In the lighter soil types (T1 and T2), egg production by the Avoyelles isolate was significantly greater than in the heaviest soil (T3). Soil type did not have a significant effect on the number of eggs per gram of root of

Evangeline and Rapides isolates. Within soil types, the only difference was that in T2 soil there was greater number of eggs per gram produced by Avoyelles isolate than the Evangeline isolate.

Table 5. Numbers of vermiform stages in soil, eggs per gram of root, and reproductive values for three isolates of *Rotylenchulus reniformis* in three soil types recovered from Stoneville LA887 in a greenhouse environment[V].

Soil type[W]	Reniform isolate[X]	Vermiform stages per 1.6 kg of soil[Y]	Eggs per gram of root[Y]	Reproductive value[Z]
T1	Avoyelles	387,631 ab	50,035 a	38.8
T1	Evangeline	219,788 bc	34,127 ab	22.0
T1	Rapides	436,038 a	31,883 ab	43.6
T2	Avoyelles	344,111 ab	52,087 a	34.4
T2	Evangeline	133,376 c	10,264 b	13.3
T2	Rapides	374,598 ab	19,477 ab	37.5
T3	Avoyelles	154,764 c	7,422 b	15.5
T3	Evangeline	117,202 c	6,891 b	11.7
T3	Rapides	234,775 bc	14,680 ab	23.5

[V]Data combined over two 60 day duration experiments with a total of 11 replications.
[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[X]Reniform nematode isolates are Avoyelles, Evangeline and Rapides, identified according to the Parish from which they were originally obtained.
[Y]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05$). Means followed by the same letter in a column are not significantly different.
[Z]Nematodes were extracted from a 500 cm^3 soil sample and converted to numbers per pot (1.6 kg of soil) in order to estimate reproductive values. Original infestation level was 10,000 vermiform stages per pot. Reproductive values (R values) were calculated as R = Pf/Pi, where Pf is the final population density and Pi is the initial infestation level.

Neither soil type nor reniform nematode isolate had a significant effect on plant height and shoot or root dry weights (Appendix, Table A2).

During the course of this experiment, the average temperature in the greenhouse was 34.2ºC in both runs of the experiment, which ranged from 28.9 to 36.7ºF in the first run and from 23.9 to 43.3ºC in the second run.
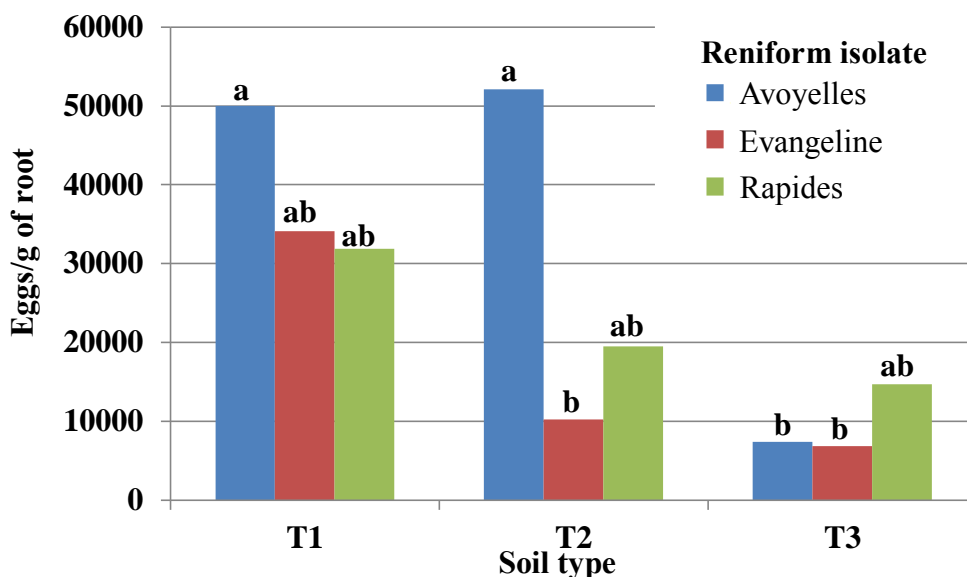
Figure 1. Individual treatment means for the interaction between soil type and reniform isolate on egg production on the cotton cultivar Stoneville LA887 in a greenhouse environment. Across all columns, means followed by the same letter do not differ significantly according to Tukey's HSD test, P ≤ 0.05.

Greenhouse - Experiment 3:

There were some significant main effects due to both soil type and reniform isolate for Stoneville 5288B2F cotton. However, the main effect for soil type were consistent across the entire duration the experiment while those associated with the reniform isolate were confined only to plant height at 15 days after inoculation (DAI) (Table 6).

Table 6. Main and interaction effects (P values) of soil type and reniform isolate on Stoneville 5288B2F cotton in a greenhouse environment[V].

Source	DF	Plant height 15 DAI[Y]	Plant height 30 DAI	Plant height 50 DAI	Root weight[Z]	Shoot weight[Z]
Soil type[W] (S)	2	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*
Reniform isolate[X] (I)	3	0.01*	0.46	0.81	0.21	0.88
S x I	6	0.16	0.09	0.03*	0.24	0.11

[V]The experimental duration was 60 days and there were six replications of each treatment. Data was analyzed with ANOVA and Tukey's HSD (P ≤ 0.05).
[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[X]Reniform nematode isolates were collected from Avoyelles, Evangeline and Rapides Parishes.
[Y]DAI = days after inoculation.
[Z]Data are dry weight obtained after two days at 45ºC.
*Indicates a significant P value.

Plants growing in T2 soil were significantly taller than those growing in either T1 or T3 soils at 15 DAI. At 50 DAI (harvest), plants growing in T1 soil had the least dry root and shoot weights.

There was a significant interaction between soil type and reniform isolate that influenced plant height at harvest (Figure 2). There was no significant difference in plant height within each of the soil types. Plants inoculated with the Evangeline and Rapides isolates were taller in T2 than in T1 soil.
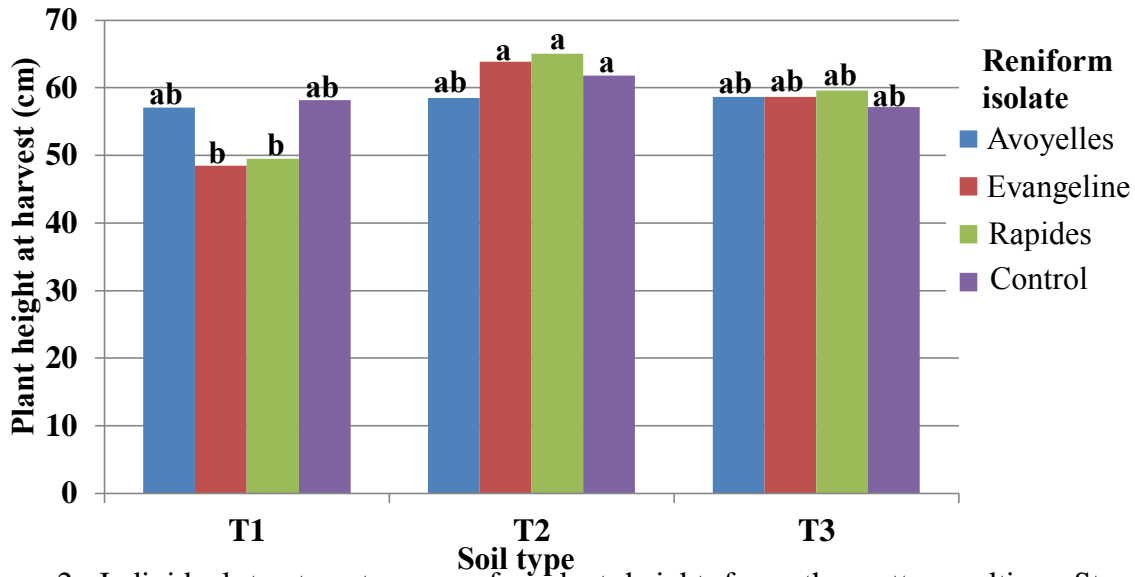
Figure 2. Individual treatment means for plant height from the cotton cultivar Stoneville 5288B2F for the interaction between soil type and reniform isolate in a greenhouse environment. Means across all columns followed by the same letter do not differ significantly according to Tukey's HSD test, $P \leq 0.05$. Control refers to the treatment that received no nematodes.

Both vermiform stages per 500 cm^3 of soil and number of eggs per gram of root were significantly affected by soil type in this experiment (Table 7). There were no significant effect of reniform isolates in this experiment, but there were significant soil type by reniform isolate interaction which influenced the number of vermiform stages in the soil. Across soil types, the Rapides isolate produced greater numbers of vermiform stages in the lightest textured soil (T1)

than in the heavier textured soils (T2 and T3) (Figure3). Also, within the T1 soil, there was

greater reproduction by the Rapides than the Evangeline isolate.

Table 7. Main and interaction effects (P values) of soil type and reniform isolate on Stoneville 5288B2F cotton in a greenhouse environment[X].

Source	DF	Vermiform stages/500 cm^3 of soil	Eggs/g of root
Soil type[Y] (S)	2	< 0.01*	< 0.01*
Reniform isolate[Z] (I)	2	0.19	0.22
S x I	4	0.01*	0.23

[X]The experimental duration was 60 days and there were six replications of each treatment. Data was analyzed with ANOVA and Tukey's HSD (P ≤ 0.05).
[Y]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[Z]Reniform nematode isolates were collected from Avoyelles, Evangeline and Rapides Parishes.
*Indicates a significant P value.
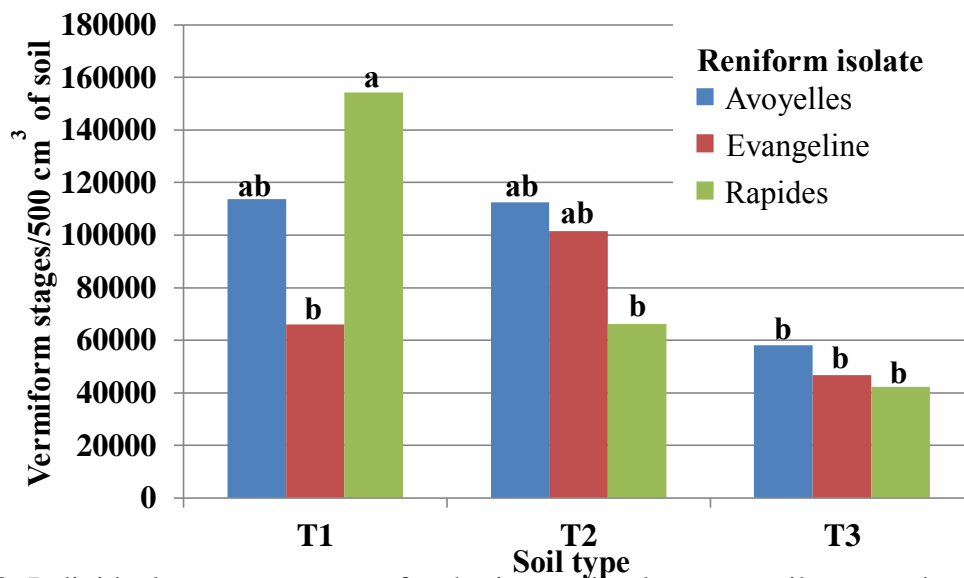
Figure 3. Individual treatment means for the interaction between soil type and reniform isolate on numbers of vermiform stages per 500 cm^3 of soil on the cotton cultivar Stoneville 5288B2F in a greenhouse environment. Across all columns, means followed by the same letter do not differ significantly according to Tukey's HSD test, P ≤ 0.05.

Among all three soil types, final population densities for the Avoyelles isolate ranged

from 186,027 to 363,520 vermiform stages per 1.6 kg of soil and eggs per gram of root ranged

from 6,864 to 27,391 (Table 8). Reproductive values for this isolate ranged from 18.6 to 36.4.

Likewise, final population densities for Evangeline isolate ranged from 135,509 to 324,864 with

egg numbers averaging from 6,783 to 14,519. Reproductive values for the Evangeline isolate ranged from 13.6 to 32.5. With the Rapides isolate, population densities ranged 149,504 to 493,909, egg numbers ranged from 3,330 to 27,237 per gram of root, and reproductive values ranging from 15.0 to 49.4. Final population densities and overall reproductive values were greatest for all three of the isolates in T1 soil.

During the course of this experiment, temperatures in the greenhouse averaged 33.4ºC, with minimum and maximum temperatures of 23.9 and 37.2ºC, respectively.

Table 8. Numbers of vermiform stages in soil, eggs per gram of root, and reproductive values for three isolates of *Rotylenchulus reniformis* in three soil types recovered from Stoneville 5288B2F in a greenhouse environment[V].

Soil type[W]	Reniform isolate[X]	Vermiform stages per 1.6 kg of soil[Y]	Eggs per gram of root[Y]	Reproductive value[Z]
T1	Avoyelles	363,520 ab	26,082 ab	36.4
T1	Evangeline	211,541 b	31,919 a	21.2
T1	Rapides	493,909 a	27,237 ab	49.4
T2	Avoyelles	360,192 ab	27,391 ab	36.0
T2	Evangeline	324,864 ab	14,519 abc	32.5
T2	Rapides	212,053 b	9,815 bc	21.2
T3	Avoyelles	186,027 b	6,864 bc	18.6
T3	Evangeline	135,509 b	6,783 bc	13.6
T3	Rapides	149,504 b	3,330 c	15.0

[V]The experimental duration was 60 days and there were six replications of each treatment.
[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[X]Reniform nematode isolates are Avoyelles, Evangeline and Rapides, identified according to the Parish from which they were originally obtained.
[Y]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05$). Means followed by the same letter in a column are not significantly different.
[Z]Nematodes were extracted from a 500 cm^3 soil sample and converted to numbers per pot (1.6 kg of soil) in order to estimate reproductive values. Original infestation level was 10,000 vermiform stages per pot. Reproductive values (R values) were calculated as R = Pf/Pi, where Pf is the final population density and Pi is the initial infestation level.

Greenhouse - Experiment 4:

Soil type significantly affected both the height and dry shoot weight of Phytogen 375WF cotton (Table 9). At 15 and 30 DAI, plants growing in T3 soil were significantly taller than

plants growing in T1 soil. Dry shoot weights in T1 soil were significantly reduced. There were

no main or interactive effects of reniform isolate on the plant parameters measured.

Table 9. Main and interaction effects (P values) of soil type and reniform isolate on Phytogen 375WF cotton in a greenhouse environment – Plant parameters[V].

Source	DF	Plant height 15 DAI[Y]	Plant height 30 DAI	Plant height 50 DAI	Root weight[Z]	Shoot weight[Z]
Soil type[W] (S)	2	0.03*	0.03*	0.49	0.46	< 0.01*
Reniform isolate[X] (I)	3	0.66	0.39	0.37	0.98	0.16
S x I	6	0.68	0.39	0.11	0.99	0.88

[V]The experimental duration was 60 days and there were six replications of each treatment. Data was analyzed with ANOVA and Tukey's HSD ($P \leq 0.05$).
[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[X]Reniform nematode isolates were collected from Avoyelles, Evangeline and Rapides Parishes.
[Y]DAI = days after inoculation.
[Z]Data are dry weight obtained after two days at 45ºC.
*Indicates a significant P value.

Both main and interaction effects of soil type and reniform isolate significantly influenced

numbers of vermiform stages per 500 cm^3 of soil and eggs per gram of root of the nematode

(Table 10). The interactive effects of soil type and reniform isolate are shown graphically as

Figures 4 and 5.

Table 10. Main and interaction effects (P values) of soil type and reniform isolate on Phytogen 375WF cotton in a greenhouse environment – Nematode density[X].

Source	DF	Vermiform stages/500 cm^3 of soil	Eggs/g of root
Soil type[Y] (S)	2	< 0.01*	< 0.01*
Reniform isolate[Z] (I)	2	< 0.01*	< 0.01*
S x I	4	< 0.01*	< 0.01*

[X]The experimental duration was 60 days and there were six replications of each treatment. Data was analyzed with ANOVA and Tukey's HSD ($P \leq 0.05$).
[Y]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[Z]Reniform nematode isolates were collected from Avoyelles, Evangeline and Rapides Parishes.
*Indicates a significant P value.

Vermiform stages per 500 cm^3 of soil for the Avoyelles isolate were significantly greater

than those for either Evangeline or Rapides isolates in T1 soil. In T2 and T3 soils, the patterns

were similar to that of T1, but there were no significant differences in nematode numbers per 500cm^3 of soil.
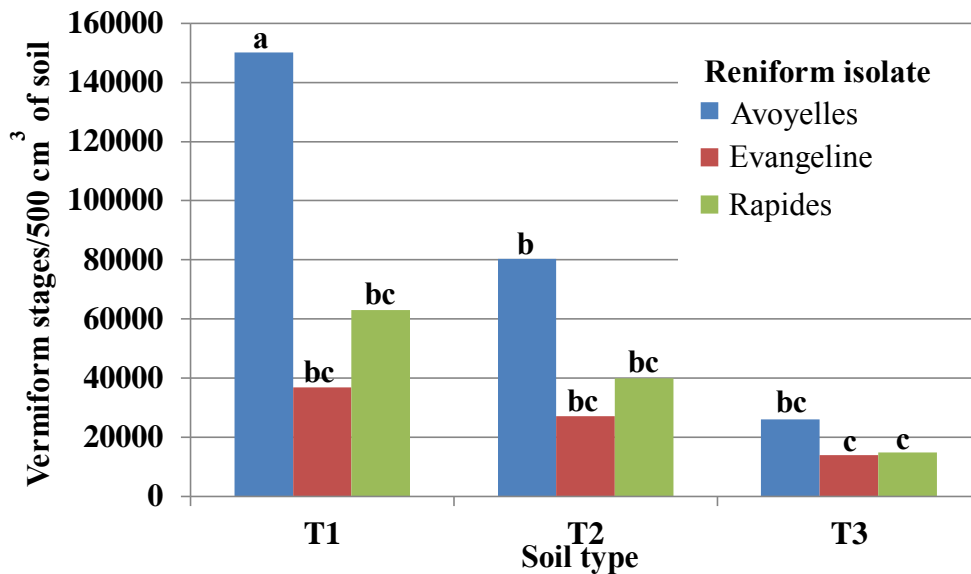
Figure 4. Individual treatment means for the interaction between soil type and reniform isolate on numbers of vermiform stages per 500 cm^3 of soil on Phytogen 375WF cotton in a greenhouse environment. Across all columns, means followed by the same letter do not differ significantly according to Tukey's HSD test, P ≤ 0.05.

Numbers of eggs per gram of root followed a trend similar to that observed for vermiform stages per 500 cm^3 of soil. For all three isolates, there were significantly more eggs per gram of root in T1 soil than in T2 and T3 soils (Figure 5, and Table 11). Additionally, in T1 soil, the number of eggs per gram of root for the Avoyelles isolate was three times greater than those produced in T2 soil, and almost six times greater than those produced in T3.

Final population densities of Avoyelles ranged from 480,427 to 83,200 vermiform stages per 1.6 kg of soil, with average number of eggs per gram of root ranging from 4,385 to 60,070. Reproductive values for this isolate ranged from 8.3 to 48.0. Similarly, Evangeline isolate final population densities ranged from 117,760 to 44,800 and the average number of eggs ranged from 1,335 to 22,801. Reproductive values of Evangeline isolate ranged from 4.5 to 11.8. Finally, Rapides had final populations ranging from 201,600 to 47,787, egg numbers ranging from 1,771

to 41,186, and R values from 4.8 to 20.2 (Table 11). Temperatures in the greenhouse during the course of this experiment averaged 34ºC, ranging from 23.9 to 40.6ºC.
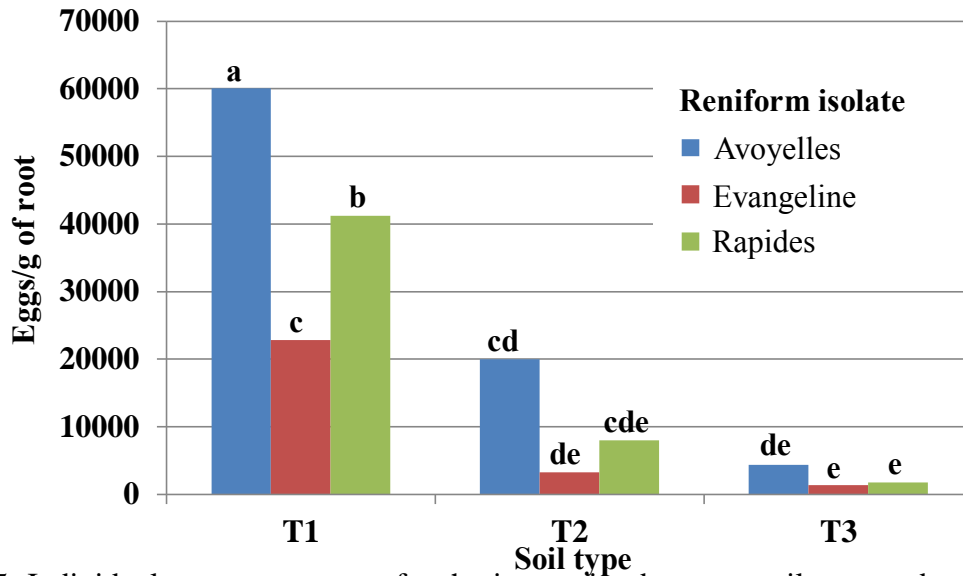
Figure 5. Individual treatment means for the interaction between soil type and reniform isolate on egg production on the cotton cultivar Phytogen 375WF in a greenhouse environment. Across all columns, means followed by the same letter do not differ significantly according to Tukey's HSD test, P ≤ 0.05.

Microplot Experiment:

Soil types significantly influenced plant height at 75 DAI and root and shoot dry weights at harvest, as well as number of opened bolls and cotton seed weight. The nematode significantly influenced number of bolls opened and cotton seed weight. There was no significant interaction between soil type and nematode (Table 12).

Numbers of vermiform stages per 500 cm^3 of soil were significantly affected by soil type and there was significant soil type by nematode interaction which influenced the number of vermiform stages in soil (Table 13). The fine texture of T2 soil resulted in reniform population densities at harvest that averaged just over two million vermiform stages per microplot, with respective reproductive value averaging 62.1. In the heavier textured soil (T3), final population

densities averaged 743,040 individuals per microplot and yielded reproductive values of only

24.8 (Table 14).

Table 11. Numbers of vermiform stages in soil, eggs per gram of root, and reproductive values for three isolates of *Rotylenchulus reniformis* in three soil types recovered from Phytogen 375WF in a greenhouse environment[V].

Soil type[W]	Reniform isolate[X]	Vermiform stages per 1.6 kg of soil[Y]	Eggs per gram of root[Y]	Reproductive value[Z]
T1	Avoyelles	480,427 a	60,070 a	48.0
T1	Evangeline	117,760 bc	22,801 c	11.8
T1	Rapides	201,600 bc	41,186 b	20.2
T2	Avoyelles	257,280 b	19,993 cd	25.7
T2	Evangeline	87,040 bc	3,235 de	8.7
T2	Rapides	128,000 bc	8,004 cde	12.8
T3	Avoyelles	83,200 bc	4,385 de	8.3
T3	Evangeline	44,800 c	1,335 e	4.5
T3	Rapides	47,787 c	1,771 e	4.8

[V]The experimental duration was 60 days and there were six replications of each treatment.
[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[X]Reniform nematode isolates are Avoyelles, Evangeline and Rapides, identified according to the Parish from which they were originally obtained.
[Y]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05$). Means followed by the same letter in a column are not significantly different.
[Z]Nematodes were extracted from a 500 cm^3 soil sample and converted to numbers per pot (1.6 kg of soil) in order to estimate reproductive values. Original infestation level was 10,000 vermiform stages per pot. Reproductive values (R values) were calculated as R = Pf/Pi, where Pf is the final population density and Pi is the initial infestation level.

The average air temperature during the course of the microplot experiments was 32.8ºC

with maximum of 38.9ºC and minimum of 17.8ºC in the first run, and average of 31.7ºC,

maximum of 38.9ºC, and minimum of 14.4ºC in the second run. Soil temperatures averaged

29.1 ºC and 27.8 ºC in the first and second runs, respectively.

Table 12. Main and interaction effects (P values) of soil type and nematode on Stoneville LA887 cotton in a microplot environment – Plant parameters[V].

Source	DF	Plant height 30[X]	Plant height 75[X]	Plant height 140[X]	Root weight[Y]	Shoot weight[Y]	Average leaf area[Z]	Average internode length	Number of interno-des	Number of bolls opened	Number of bolls closed	Cotton seed weight
Soil type[W] (S)	2	0.49	0.05*	0.38	0.01*	< 0.01*	0.65	0.32	0.34	0.02*	0.30	< 0.01*
Nema-tode (N)	1	0.62	0.49	0.58	0.60	0.24	0.54	0.30	0.39	0.04*	0.23	0.01*
S x N	2	0.33	0.73	0.07	0.20	0.44	0.21	0.66	0.31	0.37	0.10	0.51

[V]Data combined over two 150-152 days duration experiments with three replications each. Data was analyzed with ANOVA and LSD ($P \leq 0.05$).
[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.
[X]30, 75 and 140 indicate number of days after inoculation.
[W]Data are dry weight obtained after seven days at 45ºC.
[Z]Six fully developed leaves were collected from the midpoint of each plant and used to calculate the leaf area using Assess 2.0 Image Analysis Software.
*Indicates a significant P value.

Table 13. Main and interaction effects (P values) of soil type and nematode on Stoneville LA887 cotton in a microplot environment – Nematode density[Y].

Source	DF	Vermiform stages / 500cm^3 of soil	Eggs/ 5g of root
Soil type[Z] (S)	2	0.05*	0.50
Nematode (N)	1	< 0.01*	< 0.01*
S x N	2	0.05*	0.12

[Y]Data combined over two 150-152 days duration experiments with three replications each. Data was analyzed with ANOVA and LSD (P ≤ 0.05).

[Z]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.

*Indicates a significant P value.

Table 14. Numbers of vermiform stages in soil, eggs per gram of root, and reproductive values for three isolates of *Rotylenchulus reniformis* in three soil types recovered from Stoneville LA887 in a microplot environment[W].

Soil type[X]	Vermiform stages per 27.3 kg of soil[Y]	Eggs per 5 g of root[Y]	Reproductive value[Z]
T1	1,863,360 ab	32,000 a	62.1
T2	2,151,360 a	53,433 a	71.7
T3	743,040 b	21,133 a	24.8

[W]Data combined over two 150-152 days duration experiments with a total of six replications.

[X]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.

[Y]Data analyzed with ANOVA and LSD test (P ≤ 0.05). Means followed by the same letter in a column are not significantly different.

[Z]Nematodes were extracted from a 500 cm^3 soil sample and converted to numbers per microplot (27.3kg of soil) in order to estimate reproductive values. Original infestation level was 30,000 vermiform stages per pot. Reproductive values (R values) were calculated as R = Pf/Pi, where Pf is the final population density and Pi is the initial infestation level.

DISCUSSION

During this research, greenhouse experiments and microplot experiments were performed to evaluate the influence of soil texture on reproduction and pathogenicity of *R. reniformis* in cotton.

Soil type influenced plant height of Stoneville LA887 in experiment 1 but not in experiment 2. Plants growing in field soil with 31.4% sand, in experiment 1, were taller than those in soil with 72.1% of sand. In the third greenhouse experiment, with Stoneville 5288B2F, soil type significantly influenced all plant parameters measured. The greatest reduction in plant height and dry weights for Stoneville 5288B2F cultivar occurred in the lightest textured soil, T1. In experiment 4, with Phytogen 375WF, T1 soil again had the greatest impact on plant growth. However, there were no differences in the final 50 day plant height. Observations such as this, where plant height is reduced early in the season and diminishes over time, are not uncommon under field conditions (C. Overstreet, pers. comm.).

This research clearly demonstrates the impact of soil texture on the reproduction of reniform nematode. In both greenhouse and microplot environments, soil texture had a significant effect on population density of *R. reniformis*. Previous studies have also shown that soil texture has significantly influenced reproduction of reniform nematode (Robinson et al., 1987; Koenning et al., 1996; Overstreet et al., 2010; Moore and Lawrence, 2011; Overstreet et al., 2011a). In the heaviest textured soil, herein referred to as T3, nematode reproduction was significantly less than those in finer textured soils, referred to as T1 and T2.

In the first greenhouse experiment, with the Avoyelles isolate and Stoneville LA887 cotton, there was no difference in nematode reproduction across the two soil types used. In the second greenhouse experiment, again with Stoneville LA887 cotton and including the three isolates of

reniform nematode from Avoyelles, Evangeline, and Rapides Parishes, the lowest numbers of vermiform stages in 500 cm^3 of soil and eggs per gram of root were associated with the heavier T3 soil.

Among the three cotton cultivars, the greatest reproduction of all the reniform isolates was observed in the lighter textured soils (T1 and T2). Nematode reproduction was primarily governed by soil type, but the overall magnitude was determined by the cultivar. In the cultivar Stoneville 5288B2F the Rapides isolate reached the greatest nematode population density in T1 soil but in the cultivar Phytogen 375WF, the Avoyelles isolate was the one with the greatest final population density in the same soil type.

In all four greenhouse experiments, the lighter textured soils (T1 and T2) might have produced the most favorable environment for motility of the nematode, probably as a result of their water holding capacity and aeration characteristics. Within T1 and T2 soils, the magnitude of nematode reproduction was governed by both cotton cultivar and nematode isolate. The particle size distribution of T1 soil (Table 1) employed in this research is similar to the Portsmouth loamy sand soil (72% sand, 18% silt, and 10% clay) used by Herring et al. (2010) and Koenning et al. (1996). Portsmouth loamy sand and T1 soils were the soil types in which significantly greater nematode reproduction occurred when compared with the other soil types. Also, Herring et al. (2010) observed the lowest nematode reproduction on two soil types having 29% and 39% of clay. These observations were similar to data for the T3 soil type, with 25.9% clay, which showed less reproduction of reniform nematode.

Across both cotton cultivars and reniform isolates employed in experiments 2, 3, and 4, T1 soil provided the environment for the greatest amount of vermiform stages per 500 cm^3 of soil and eggs per gram of root. Reproduction values in T1 soil ranged from 26.7 on Phytogen 375WF

29

to 35.7 on Stoneville 5288B2F. In T2 soil, reproduction values ranged from 15.7 in Phytogen 375WF to 29.9 in Stoneville 5288B2F. Number of eggs per gram of root followed the same trend, in which the number of eggs was greater in T1 than in T2 soil. In T1 soil, eggs per gram of root ranged from 28,413 in Stoneville 5288B2F to 41,356 in Phytogen 375WF. In T2 soil, the number of eggs per gram varied from 10,411 in Phytogen 375WF to 27,276 in Stoneville LA887.

Across the three cotton cultivars, soil type had less of an impact on growth than it did on reproduction of the nematode. Cook et al. (1997) also reported a lack of interaction between reniform nematode and plant height and dry shoot weight in a 12-week duration greenhouse experiment.

In the microplot experiment, plant height at 75 DAI, approximately the midpoint of the experiment, and dry root and shoot weights at harvest were significantly affected by soil type, with taller plants and greater dry weights recorded from plants growing in the heaviest soil (T3).

In the microplot environment, the greater reproduction of nematodes was observed in T1 and T2 soils. In these soils, the numbers of bolls opened and cotton seed weights were significantly reduced by the nematode.

Observations in the microplot experiment revealed that cotton maturity was delayed by reniform nematode due to the significant reduction in number of bolls opened. Consequently, this delay produced a significant reduction in seed cotton weight. Similar results were obtained previously by Koenning et al. (1996) in North Carolina, while studying the impact of soil texture on the reproductive and damage potential of *R. reniformis* on cotton.

The research reported in here supports, to a limited extent, previous reports of variation in pathogenicity among reniform isolates (McGawley et al., 2010). This variation was evidenced by

significant reduction in cotton plant dry weights observed in some greenhouse experiments and also as reduction of seed cotton weight in the microplot experiment.

While studying the effects of soil texture on *Heterodera glycines* on soybeans, Koenning et al. (1988) considered that there is an optimum limit in sand content that will favor the reproduction of *Heterodera* species. As reported in previous studies, *R. reniformis* is favored by fine textured soils (Robinson et al., 1987; Koenning et al., 1996; Overstreet et al., 2011a). However, it is noticeable in the present research that there is also an optimum of clay content that will limit the reproduction of the reniform nematode. Koenning et al. (1996) and Xavier et al. (2012) have reported similar observations in microplot and field experiments, where population densities of reniform nematode were greater in samples with clay content ranging from 18% to 20%. However, as clay content increases above 20%, there is a limitation in *R. reniformis* reproduction.

For the three nematode isolates tested, a reduction in nematode reproduction is evident with the increase in clay content. The marked differences in population densities, observed in Stoneville 5288B2F and Phytogen 375WF cultivars are noticeable in T1 soil, but they decrease considerably in T2 soil and almost disappear in T3 soil. These observations re-emphasize the fact that there is a limit to the influence of clay content on population development of *R. reniformis*.

Monfort et al. (2008) reported an effect of silt content on reproduction and spread of reniform nematode in a field over years, where the nematode seems to establish itself better in soils with silt contents ranging from 51% to 68%. Moore and Lawrence (2011) have also reported greater nematode reproduction in soils with silt and clay contents ranging from 49% to 42%, and 28% to 53%, respectively, in microplot experiments in Alabama. In the microplot phase of this research, the greatest nematode reproduction was observed in soils with about 55%

silt and 13% clay. This observation is in opposition to the microplot data of Moore and Lawrence (2011). Environmental factors, such as soil formation from different parental materials (Petersen and Calvin, 1986) and differences in geographic isolates of reniform nematode (McGawley et al., 2010) might also have contributed for the divergence of results among these two reports.

SUMMARY

This research focused on the impact of soil texture on reproduction and pathogenicity of *R. reniformis* on cotton growing in greenhouse and microplot environments. Major variables in this research were: isolate of reniform nematode, cotton cultivars, and soil textures. Overall, soil texture had a greater impact on nematode reproduction than it did on cotton growth parameters. Commerce silt loam soils with clay content greater than 20% tends to restrict reproduction of *R. reniformis*. Isolates of reniform nematode were significantly impacted by soil type and cotton cultivars. Such information is essential for refining management zones in cotton production areas where reniform nematode is the predominant pathogen.

REFERENCES

Agudelo, P., R. T. Robbins, J. Mcd. Steward, and A. L. Szalanski. 2005. Intraspecific variability of *Rotylenchulus reniformis* from cotton-growing regions in the United States. Journal of Nematology 37: 105-114.

Agudelo, P. 2007. Reniform nematode (*Rotylenchulus reniformis*) research in the United States. Nematropica 37: 135.

American Society for Testing and Materials. 1985. Standard test method for particle-size analysis of soils. 1985 Annual Book of ASTM Standards pp 117-127. American Society for Testing and Materials, Philadelphia.

Anonymous. 2010a. Crop production 2009 Summary-January 2010. United States Department of Agriculture, National Agricultural Statistics Service website. http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2010.pdf (Accessed: 8 February 2011).

Anonymous. 2010b. Crop production 2009 Summary-January 2010. United States Department of Agriculture, National Agricultural Statistics Service website. http://usda.mannlib.cornell.edu/usda/current/CropValuSu/CropValuSu-02-19-2010.pdf (Accessed: 8 February 2011).

Anonymous. 2011a. Cotton varieties planted 2011 crop. United States Department of Agriculture, Agricultural Marketing Service website. http://www.ams.usda.gov/mnreports/cnavar.pdf (Accessed: 12 February 2012).

Anonymous. 2011b. SST Software website. http://www.sstsoftware.com/sstoolbox.htm (Accessed: 18 January 2011).

Anonymous. 2013. Crop production 2012 Summary-January 2013. United States Department of Agriculture, National Agricultural Statistics Service website. http://usda01.library.cornell.edu/usda/current/CropProdSu/CropProdSu-01-11-2013.pdf (Accessed: 2 February 2013).

Avendaño, F., F. J. Pierce, and H. Melakeberhan. 2004a. Spatial analysis of soybean yield in relation to soil texture, soil fertility and soybean cyst nematode. Nematology 6: 527-545.

Avendaño, F., F. J. Pierce, and H. Melakeberhan. 2004b. The relationship between soybean cyst nematode seasonal population dynamics and soil texture. Nematology 6: 511-525.

Avendaño, F., F. J. Pierce, O. Schanbenberger, and H. Melakeberhan. 2004c. The spatial distribution of soybean cyst nematode in relation to soil texture and soil map unit. Agronomy Journal 96: 181-194.

Barker, K. R., and W. W. Weeks. 1981. Influence of soil type and *Meloidogyne incognita* on yield and quality of tobacco. Journal of Nematology 13: 432.

Barker, K. R., and S. R. Koenning. 1998. Developing sustainable systems for nematode management. Annual Review of Phytopathology 36: 165-205.

Birchfield, W., and J. E. Jones. 1961. Distribution of the reniform nematode in relation to crop failure of cotton in Louisiana. Plant Disease Reporter 45: 671-673.

Birchfield, W. 1962. Host parasite relations of *Rotylenchulus reniformis* on *Gossypium hirsutum*. Phytopathology, 52: 862-865.

Blasingame, D., J. C. Banks, P. D. Colyer, R. M. Davis, W. S. Gazaway, N. Goldburg, R. C. Kemerait, T. L. Kirkpatrick, S. R. Koenning, J. Muller, M. A. Newman, M. Olsen, P. M. Phipps, G. L. Sciumbato, R. Sprenkel, J. E. Woodward, A. Wrather, and M. V. Patel. 2008. Cotton disease loss estimate committee report. Proceedings of the Beltwide Cotton Conferences; 8-11 January 2008; Nashville, TN. National Cotton Council, Cordova, TN. Pp. 294–297.

Blasingame, D., and M. V. Patel. 2012. Cotton disease loss estimate committee report. Proceedings of the Beltwide Cotton Conferences; 3-6 January 2012; Orlando, FL. National Cotton Council, Cordova, TN. Pp. 341-344.

Byrd, D. W., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small and C. A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8: 206-212.

Cabanillas, H. E., J. M. Bradford, and J. R. Smart. 1999. Effect of tillage system, soil type, crop stand, and crop sequence on reniform nematodes after harvest. Nematropica 29: 137-146.

Cook, C. G., A. F. Robinson, and L. N. Namken. 1997. Tolerance to *Rotylenchulus reniformis* and resistance to Meloidogyne incognita race 3 in high-yielding breeding lines of upland cotton. Journal of Nematology 29: 322-328.

Davis, R. F., S. R. Koenning, R. C. Kemerait, T. D. Cummings, and W. D. Shurley. 2003. *Rotylenchulus reniformis* management in cotton with crop rotation. Journal of Nematology 35: 58-64.

Day, P. R. 1965. Particle fractionation and particle-size analysis. In C. A. Black et al. (ed.) Methods of soil analysis, Part I. Agronomy, 9: 545-567.

Erwin, T., R. Letlow, C. Overstreet, and M. Wolcott. 2007. Nematode management of cotton in Morehouse Parish, Louisiana. Proceedings of the Beltwide Cotton Conferences; 9-12 January, 2007; New Orleans, LA. National Cotton Council, Cordova, TN. Pp.1368-1376.

Erwin, T. L., R. M. Letlow, and C. Overstreet. 2010. Seed treatment nematicides against root-knot nematode in Northeast Louisiana. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2010; New Orleans, LA. National Cotton Council, Cordova, TN. Pp. 184-185.

Evans, K., R. Webster, A. Barker, P. Halford, and M. Russell. 2003. Mapping infestations of potato cyst nematodes and the potential for spatially varying application of nematicides. Precision Agriculture 4: 149-162.

Faske, T. R., and J. L. Starr. 2005. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Abamectin. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2005; New Orleans, LA. National Cotton Council, Cordova, TN. P. 180.

Gazaway, W. 2005. Nematodes: what we know and what we are doing. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2005; New Orleans, LA. National Cotton Council, Cordova, TN. P. 310.

Griffin, G. D. 1996. Importance of soil texture to the pathogenicity of plant-parasitic nematodes on rangeland grasses. Nematropica 26: 27-37.

Herring, S. L., S. R. Koenning, J. L. Heitman. 2010. Impact of *Rotylenchulus reniformis* on cotton yield as affected by soil texture and irrigation. Journal of Nematology 42: 319-323.

Hewlett T. E., S. R. Stetina, L. M. Schmidt. 2010. Rate of germination and growth of *in vitro* produced *Pasteuria* spp. parasitizing *Rotylenchulus reniformis*. Journal of Nematology 42: 247-248.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods for collecting inocula for *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57: 1025-1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48: 692.

Koenning, S. R., S. C. Anand, and J. A. Wrather. 1988. Effect of within-field variation in soil texture on *Heterodera glycines* and soybean yield. Journal of Nematology 20: 373-380.

Koenning, S. R., S. A. Walters, and K. R. Barker. 1996. Impact of soil texture on the reproductive and damage potentials of *Rotylenchulus reniformis* and *Meloidogyne incognita* on cotton. Journal of Nematology 28: 527-536.

Koenning, S. R., T. L. Kirkpatrick, J. L. Starr, J. A. Wrather, N. R. Walker, and J. D. Mueller. 2004. Plant-parasitic nematodes attacking cotton in the United States. Plant Disease 88: 100-113.

Linford, M. B., and J. M. Oliveira. 1940. *Rotylenchulus reniformis*, nov. gen. n. sp., a nematode parasite of roots. Proceeding of the Helminthological Society of Washington 7: 35-42.

McGawley, E. C., M. J. Pontif, J. Bruce, and C. Overstreet. 2006. Efficacy of Agri-Terra against nematodes associated with major crop species in Louisiana. Journal of Nematology 38:282.

McGawley, E. C., M. J. Pontif and C. Overstreet. 2010. Variation in reproduction and pathogenicity of geographic isolates of *Rotylenchulus reniformis* on cotton. Nematropica 40: 275-288.

McGawley, E. C., M. J. Pontif and C. Overstreet. 2011. Variation in reproduction and pathogenicity of geographic isolates of *Rotylenchulus reniformis* on soybean. Nematropica 41: 12-22.

McGawley, E. C., C. Overstreet and M. J. Pontif. 2012. Variation in eclosion and hatch of eggs among geographic isolates of *Rotylenchulus reniformis*. Journal of Nematology, *in press*.

Melakeberhan, H. 2002. Embracing the emerging precision agriculture technologies for site-specific management of yield-limiting factors. Journal of Nematology 34: 185-188.

Monfort, W. S., T. L. Kirkpatrick, C. S. Rothrock, A. Mauromoustakos. 2007. Potential for site-specific management of *Meloidogyne incognita* in cotton using soil textural zones. Journal of Nematology 39: 1-8.

Monfort, W. S., T. L. Kirkpatrick, and A. Mauromoustakos. 2008. Spread of *Rotylenchulus reniformis* in an Arkansas cotton field over a four-year period. Journal of Nematology 40: 161-166.

Moore, S. R., and K. S. Lawrence. 2011. Effects of soil type on the reproductive potential of *Rotylenchulus reniformis* on cotton. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2011; Atlanta, GA. National Cotton Council, Cordova, TN. Pp. 235-240.

National Cotton Council. 2010. Beltwide yield losses to nematode damage. [Online]. Available at http://www.cotton.org/tech/pest/nematode/losses.cfm (Accessed: 19 November 2010).

Nichols, R. L., A. Bell, D. Stelly, N. Dighe, A.F. Robinson, M. Menz, J. Starr, D. Keim, J. Jones, C. Overstreet, D. Fang, and R. Lemon. 2010. Phenotypic and genetic evaluation of LONREN germplasm. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2010; New Orleans, LA. National Cotton Council, Cordova, TN. Pp. 798-799.

Ortiz, B. V., D. Sullivan, C. Perry, and G. Vellidis. 2007. Geostatistical analysis of the spatial variability of cotton-parasitic nematodes and the factors favoring its occurrence. Proceedings of the Beltwide Cotton Conferences; 9-12 January, 2007; New Orleans, LA. National Cotton Council, Cordova, TN. Pp. 920-928.

Ortiz, B. V., C. Perry, D. Sullivan, P. Lu, R. Kemerait, R. F. Davis, A. Smith, G. Vellidis, and R. Nichols. 2012. Variable rate application of nematicides on cotton fields: a promising site-specific management strategy. Journal of Nematology 44: 31-39.

Overstreet, C., and E. C. McGawley. 1996. Current incidence of plant parasitic nematodes in Louisiana. Proceedings of the Beltwide Cotton Conferences; 9-12 January, 1996; Nashville, TN. National Cotton Council, Cordova, TN. Pp. 253-254.

Overstreet, C., and E. C. McGawley. 1998. The emergence of *Rotylenchulus reniformis* as the major nematode pest in Louisiana, U.S.A. Nematropica 28: 142.

Overstreet, C. and E. C. McGawley. 2000. Geographical dispersion of reniform nematode in Louisiana. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2000; San Antonio, TX. National Cotton Council, Cordova, TN. Pp. 168-170.

Overstreet, C., E. Burris, D. R. Cook, E. C. McGawley, B. Padgett, and M. Wolcott. 2007. Telone II Fumigation. Proceedings of the Beltwide Cotton Conferences; 9-12 January, 2007; New Orleans, LA. National Cotton Council, Cordova, TN. Pp. 587-598.

Overstreet, C. and M. Wolcott. 2007. Diagnosing reniform nematode problems: Telone II Fumigation. Proceedings of the Beltwide Cotton Conferences; 9-12 January, 2007; New Orleans, LA. National Cotton Council, Cordova, TN. Pp. 15-22.

Overstreet C., E. C. McGawley, G. Burris, and D. Burns. 2010. Site-specific management strategies for dealing with multiple nematode pests. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2010; New Orleans, LA. National Cotton Council, Cordova, TN. Pp. 305-311.

Overstreet C., E. C. McGawley, D. Burns, and R. L. Fraizer. 2011a. Edaphic factors involved in the delineation of management zones for *Rotylenchulus reniformis*. Journal of Nematology 43: 268.

Overstreet, C., R. Barbosa, D. Burns, R. L. Frazier, E. C. McGawley, G. B. Padgett, and M. C. Wolcott. 2011b. Using electrical conductivity to determine nematode management zones in alluvial soils of the mid-South. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2011; Atlanta, GA. National Cotton Council, Cordova, TN. Pp. 252-258.

Parkhi, V., V. Kumar, L. M. Campbell, A. A. Bell, J. Shah, and K. S. Rathore. 2010. Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing Arabidopsis NPR1. Transgenic Research 19: 959-975.

Perry, C., G. Vellidis, D. Sullivan, K. Rucker, and R. C. Kemerait. 2006. Predicting nematode hotspots using soil EC data. Proceedings of the Beltwide Cotton Conferences; 3-6 January, 2006; San Antonio, TX. National Cotton Council, Cordova, TN. Pp. 502-511.

Petersen, R. G., and L. D. Calvin. 1986. Sampling. In Arnold Klute (ED.), Methods of Soil Analysis Part 1 – Physical and Minerological Methods. American Society of Agronomy, Inc. Madison, WI. Pp. 33-51.

Robinson, A. F., C. M. Heald, S. L. Flanagan, W. H. Thames, and J. Amador. 1987. Geographical Distributions of *Rotylenchulus reniformis, Meloidogyne incognita,* and *Tylenchulus semipenetrans* in the Lower Rio Grande Valley as Related to Soil Texture and Land Use. Annals of Applied Nematology 1: 20-25.

Robinson, A. F., and A. E. Percival. 1997. Resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico. Supplement of the Journal of Nematology 29: 746-755.

Robinson, A. F., R. N. Inserra, E. P. Caswell-Chen, N. Vovlas, and A. Troccoli. 1997. *Rotylenchulus* species: identification, distribution, host ranges, and crop resistance. Nematropica 27: 127-180.

Robinson, A. Forest. 2007. Reniform in U.S. cotton: when, where, why, and some remedies. Annual Rev. Phytopathology 45: 263-288.

Romano, G. B., E. J. Sacks, S. R. Stetina, A. F. Robinson, D. D. Fang, O. A. Gutierrez, and J. A. Scheffler. 2009. Identification and genomic location of a reniform nematode (*Rotylenchulus reniformis*) resistance locus (*Ren^{ari}*) introgressed from Gossypium aridum into upland cotton (*G. hirsutum*). Theorical and Applied Genetics 120: 139-150.

Royal, J., and G. Hammes. 2005. Effect of crop rotation, at plant and foliar nematicides on cotton yield and reniform nematode populations. A seven year summary of results in Southwest Georgia. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2005; New Orleans, LA. National Cotton Council, Cordova, TN. P. 181.

Shane, W. W. and K. R. Barker. 1986. Effects of temperature, plant age, soil texture, and *Meloidogyne incognita* on early growth of soybean. Journal of Nematology 18: 320-327.

Sikkens, R. B., A. A. Bell, T. A. Wheeler, C. Overstreet, D. B. Weaver, K. S. Lawrence, and R. L. Nichols. 2012. Performance evaluation of LONREN and BARBREN reniform nematode resistant germoplasm lines. Proceedings of the Beltwide Cotton Conferences; 3-6 January, 2012; Orlando, FL. National Cotton Council, Cordova, TN. Pp. 761-767.

Sivakumar, C. V. and A. R. Seshadri. 1972. Effect of soil texture on the reniform nematode, *Rotylenchulus reniformis*. Indian Journal of Nematology 2: 83-86.

Smith, A. L. and A. L. Taylor. 1941. Nematode distribution in the 1940 regional cotton wilt plots. Phytopathology 31: 771.

Starr J. L., S. R. Koenning, T. L. Kirkpatrick, A. F. Robinson, P. A. Roberts, and R. L. Nichols. 2007. The future of nematode management in cotton. Journal of Nematology 39: 283-294.

Stetina, S. R., L. D. Young, W. T. Pettigrew, and H. A. Bruns. 2007. Effect of corn-cotton rotations on reniform nematode populations and crop yield. Nematropica 37: 237-248.

Thomas, C. S., P. W. Skinner, A. D. Fox, C. A. Greer, and W. D. Gubler. 2002. Utilization of GIS/GPS-Based Information Technology in Commercial Crop Decision Making in California, Washington, Oregon, Idaho, and Arizona. Journal of Nematology 34: 200-206.

Walters, A. S., and K. R. Barker. 1994. Efficacy of *Paecilomyces lilacinus* in suppressing *Rotylenchulus reniformis* on tomato. Supplement to Journal of Nematology 26: 600-605.

Wang, K., R. D. Riggs, and D. Crippen. 2005. Isolation, selection, and efficacy of *Pochonia chlamydosporia* for control of *Rotylenchulus reniformis* on cotton. Phytopathology 95: 890-893.

Weaver, D. B., K. S. Lawrence, and E. van Santen. 2007. Reniform nematode resistance in upland cotton germplasm. Crop Science 47: 19-24.

Wyse-Pester D. Y., L. J. Wiles, and P. Westra. 2002. The potential for mapping nematode distributions for site-specific management. Journal of Nematology 34:80–87.

Xavier, D. M., C. Overstreet, M. T. Kularathna, C. M. Martin, D. Burns, and R. L. Fraizer. 2012. Reniform nematode (*Rotylenchulus reniformis*) development across the variable soil texture in a Commerce Silt Loam field. Proceedings of the Beltwide Cotton Conferences; 3-6 January, 20012 Orlando, FL. National Cotton Council, Cordova, TN. Pp. 242-246.

Zhao, Q., R. T. Robbins and J. McD. Stewart. 2000. Effect of different soil types on the reproduction of reniform nematode. Proceedings of the Beltwide Cotton Conferences; 4-7 January, 2000; San Antonio, TX. National Cotton Council, Cordova, TN. Pp. 173-174.
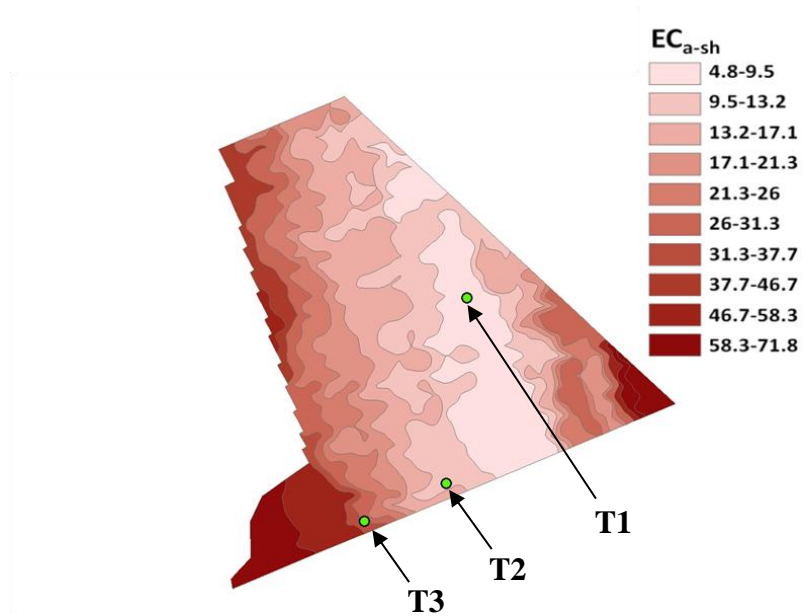
Figure A1. The Gin Ridge field located on the Northeast Research Station at St. Joseph, LA that has been divided into 10 different zones based on shallow readings (0-0.3 m) of apparent electrical conductivity (EC_{a-sh}). The sample sites where soil was collected for additional experimentation are represented by the dots in the map.
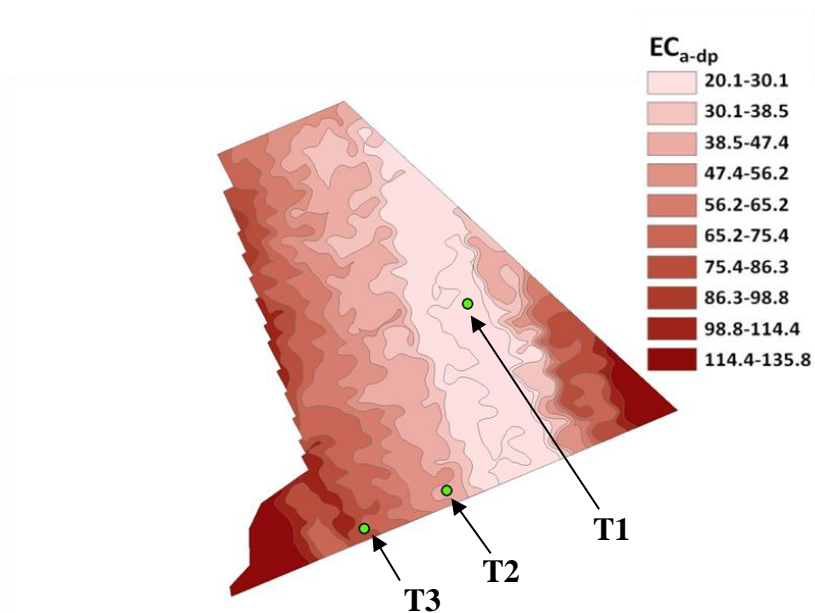

Figure A2. The Gin Ridge field located on the Northeast Research Station at St. Joseph, LA that has been divided into 10 zones based on deep readings (0-0.9 m) of apparent electrical conductivity (EC_{a-d}). The sample sites where soil was collected for additional experimentation are represented by the dots in the map.
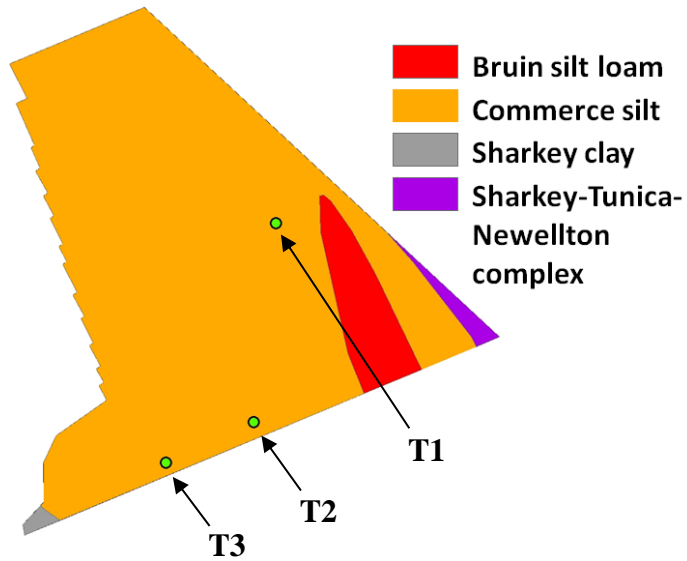
Figure A3. Soil series of the Gin Ridge field located on the Northeast Research Station at St. Joseph, LA. The sampling sites that were used for soil collection are all found in the Commerce silt loam.

Table A1. Reproduction of *Rotylenchulus reniformis* (Avoyelles isolate) on Stoneville LA887 cotton growing in two different soil types in a greenhouse environment[W].

Soil type[X]	Vermiform stages / 500cm^3 of soil[Y]	Eggs/ g of root[Y]	Reproductive value[Z]
Greenhouse	8952 a	24357 a	1.8
Field	9716 a	14440 a	2.0

[W]Data combined over two 45 day duration experiments with five replications each.

[X]Two soil lots were used in this experiment. Percentages of sand, silt, and clay were 72.1, 25.4, and 2.5 for the greenhouse lot, and 31.4, 55.3, and 13.3 for the field lot.

[Y]Data analyzed with ANOVA and Student's t-test ($P \leq 0.05$). Means followed by the same letter in a column are not significantly different.

[Z]Nematodes were extracted from a 500 cm^3 soil sample in order to estimate reproductive values. Original infestation level was 5,000 vermiform stages per pot. Reproductive values (R values) were calculated as R = Pf/Pi, where Pf is the final population density and Pi is the initial infestation level.

Table A2. Main and interaction effects (P values) of soil type and reniform isolate on Stoneville LA887 cotton in a greenhouse environment – Plant parameters[V].

Source	DF	Plant height 15 DAI[Y]	Plant height 30 DAI	Plant height 50 DAI	Root weight[Z]	Shoot weight[Z]
Soil type[W] (S)	2	0.47	0.82	0.88	0.88	0.40
Reniform isolate[X] (I)	3	0.88	0.82	0.85	0.81	0.36
S x I	6	0.93	0.99	0.99	0.63	0.92

[V]Data combined over two 60 day duration experiments with a total of 11 replications. Data was analyzed with ANOVA and Tukey's HSD (P ≤ 0.05).

[W]Soils were from three different locations within a Commerce silt loam field. Percentages of sand, silt and clay were 74.4, 31.4, and 7.8 for T1; 20.7, 55.3, and 66.3 for T2; and 4.9, 13.3, and 25.9 for T3, respectively.

[X]Reniform nematode isolates are Avoyelles, Evangeline and Rapides, identified according to the Parish where they were originally obtained.

[Y]DAI = days after inoculation.

[Z]Data are dry weight obtained after two days at 45ºC.

VITA

Déborah Magalhães Xavier, older daughter of Manoel Joaquim Xavier Filho and Marisa Magalhães Xavier, was born in 1985 in Ponte Nova, Minas Gerais, Brazil. She graduated from Federal University of Viçosa in 2009 with a Bachelor of Science degree in agronomy engineering. In 2010, she joined Louisiana State University to work on her Master's degree under the supervision of Dr. Charles Overstreet. During her time at graduate school, she was involved in different student organizations. She was an active member of the Plant Pathology and Crop Physiology Graduate Student Association, serving as secretary, bulletin board committee leader, and scholarship committee member. She also served as the chairperson of hospitality and secretary of the Latin American Student Association (LASA). In 2011 she was invited to join the Gamma Sigma Delta (College of Agriculture Honor Society). While she was a graduate student, she attended different national and international meetings to present the findings of her research. In 2012, she received second place in the student competition in the XLIV Organization of Nematologists of Tropical America (ONTA) held in Cancun, Mexico. In 2013, she was the third place recipient of an award in a student competition in the Cotton Beltwide Conference. She will receive the Master of Science degree in plant health in May, 2013.