RESEARCH/INVESTIGACIÓN

SUSCEPTIBILITY OF GRAIN SORGHUM CULTIVARS TO *MELOIDOGYNE INCOGNITA* IN LOUISIANA, U.S.A.

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ABSTRACT

Xavier-Mis, D. M., C. Overstreet, E. C. McGawley, and V. P. Doyle. 2017. Susceptibility of grain sorghum cultivars to *Meloidogyne incognita* in Louisiana, U.S.A. Nematropica 47:86-98.

Greenhouse studies were conducted to evaluate the host suitability of cultivars of sorghum currently grown in Louisiana to *Meloidogyne incognita*. A total of 29 cultivars of grain sorghum were evaluated. Nematode reproduction was highly variable among the cultivars of sorghum tested and ranged from 1,239 to 30,440 eggs/g of root and from 1,440 to 25,208 J2/500 cm^3 soil on REV RV9782 and DeKalb DKS 53-67 cultivars, respectively. A second study was conducted to determine the influence of 10 populations of *M. incognita* from Louisiana on Dekalb DKS 53-67, REV RV9924, and REV RV9782 sorghum, rated as being susceptible, moderately susceptible, and moderately resistant, respectively. Sorghum cultivar and root-knot nematode population significantly affected nematode reproduction in a greenhouse environment. As previously determined, DEKALB DKS 53-67 was the most susceptible and REV RV9782 the least susceptible across the 10 populations of *M. incognita*. Across the three cultivars of sorghum, there was significantly greater reproduction by the *M. incognita* population from St. Landry (LARK19) than the other 9 populations. There was a significant sorghum cultivar by nematode population interaction, which influenced the number of eggs/g of root. There were significant differences in egg production among the different populations of *M. incognita* on the cultivars DEKALB DKS 53-67 and REV RV9924. Despite the great variability in reproduction and broad geographical distribution of the nematode populations, no intraspecific variation of 18S and 28S ribosomal DNA was observed among them. Egg production on the resistant cultivar REV RV9782 was similar across all populations of *M. incognita*. This research highlights the need to evaluate sorghum cultivars within a geographical location for susceptibility to *M. incognita*.

Key words: grain sorghum, *Meloidogyne incognita*, nematode populations

RESUMEN

Xavier-Mis, D. M., C. Overstreet, E. C. McGawley y V. P. Doyle. 2017. Susceptibilidad de cultivares de sorgo de grano a *Meloidogyne incognita* en Louisiana, U.S.A. Nematropica 47:86-98.

Se llevaron a cabo estudios de invernadero para evaluar la compatibilidad de los cultivares de sorgo actualmente sembrados en Louisiana a *Meloidogyne incognita*. Se evaluó un total de 29 cultivares de sorgo de grano. La reproducción de los nematodos fue altamente variable entre los cultivares de sorgo evaluados, varió de 1,239 a 30,440 huevos por gramo de raíz y de 1,440 a 25,208 juveniles por 500 cm^3 de suelo en los cultivares REV RV9782 y DeKalb DKS 53-67, respectivamente. Un segundo ensayo se llevó a cabo para determinar la influencia de 10 poblaciones de *M. incognita* en sorgo de Louisiana Dekalb DKS 53-67, REV RV9924 y REV RV9782, clasificadas como susceptibles, moderadamente susceptibles y moderadamente resistentes. El cultivar de sorgo y la población de agalladores significativamente afectó la reproducción del nematodo bajo condiciones de invernadero. Como se determinó previamente, DEKALB DKS 53-67 fue el más susceptible y REV RV9782 el menos susceptible entre las 10 poblaciones de *M. incognita*. En los tres cultivares de sorgo hubo una reproducción significativamente mayor de la población de *M. incognita* de St. Landry (LARK19) que las otras 9 poblaciones. Hubo un cultivar significativo de sorgo que por la interacción de la población de nematodos influyó en el número de huevos por gramo de raíz. Hubo diferencias significativas en la producción de huevos entre las diferentes poblaciones de *M. incognita* en los cultivares DEKALB DKS 53-67 y REV RV9924. A pesar de la gran variabilidad en la reproducción y la amplia distribución geográfica de las poblaciones de nematodos, no se observó variación intraespecífica del ADN ribosómico 18S y 28S entre ellos. La producción de huevos

en el cultivar resistente REV RV9782 fue similar en todas las poblaciones de *M. incognita*. Esta investigación resalta la necesidad de evaluar los cultivares de sorgo dentro de una ubicación geográfica por la susceptibilidad a *M. incognita*.

Palabras-clave: Sorgo de grano, *Meloidogyne incognita*, poblaciones de nematodos

INTRODUCTION

The southern root-knot nematode, *Meloidogyne incognita*, is recognized on a worldwide basis as the most important root-knot nematode species on grain sorghum, *Sorghum bicolor* (L.) Moench (McGawley and Overstreet, 1998). The host status of grain sorghum for *M. incognita* is highly controversial. Reports about sorghum suitability as a host to *M. incognita* range from resistant (Fortnum and Currin, 1988; McClure *et al*., 1999; Ribeiro *et al*., 2002) to highly susceptible (Birchfield, 1983; Babatola and Idowu, 1990). There have also been a number of reports indicating variable susceptibility of sorghum cultivars to race 3 of *M. incognita* (Netscher and Taylor, 1979; McSorley and Gallaher, 1993; Carneiro *et al*., 2006). In the southern United States, *M. incognita* race 3 is the dominant root-knot nematode present in areas where cotton has been produced (Kirkpatrick and Sasser, 1983; Davis and Timper, 2000).

Sorghum is grown in 14 states in the U.S. with Kansas and Texas producing 73% of the 3.1 million ha grown in 2015 (Anonymous, 2015b). It is used as fiber, animal feed, and also as a food source in some African countries (Babatola and Idowu, 1990). Although worldwide losses to plant-parasitic nematodes were estimated for sorghum at 6.9% by Sasser and Freckman (1987), significant yield losses to sorghum from *M. incognita* have rarely been reported in the U.S. (Koenning *et al*., 1999) or in other countries (Sharma and McDonald, 1990). Orr (1967) reported damage by *M. incognita* to sorghum in fields following cotton. The symptoms of damage from *M. incognita* to sorghum included chlorosis, stunting, stand loss, and delayed blooming with yields reduced by as much as 33%. Galls were also reported as elongate swellings, discrete knots, or swellings with root proliferation and occurring primarily on lateral but not on brace roots (Orr and Morey, 1978). Reported galling by *M. incognita* on grain sorghum ranges from very severe (Birchfield, 1983), to moderate (Babatola and Idowu, 1990) and slight to none (Ibrahim *et al*., 1993).

Orr (1967) conducted greenhouse studies with *M. incognita* and grain sorghum or cotton and determined a 15% and 40% reduction in plant weights, respectively. Thomas and Murray (1987) reported 45% yield loss in grain sorghum caused by *M. incognita* in a microplot test. Babatola and Idowu (1990) reported a stepwise reduction in grain sorghum yield with increasing levels of *M. incognita* and with losses as high as 65% to 86% on three cultivars. Davis *et al*. (2015) found that sweet sorghum (*Sorghum bicolor*) cultivars were highly tolerant of *M. incognita* and not significantly damaged by this nematode.

Field populations of *M. incognita* have been shown to differ in their virulence and host ranges (Ehwaeti *et al*., 1999; Trudgill and Blok, 2001; Anwar and McKenry, 2007; Adam *et al*., 2014). Trudgill and Blok (2001) suggested that the differences observed within field populations of *Meloidogyne* spp. may not be the result of known selection pressure. However, when resistant plants have been utilized, progressive changes in the virulence of the nematode population have been observed (Roberts *et al*.,1995; Kaloshian *et al*.,1996; Zhou *et al*., 2000). Cotton and soybean cultivars that have some resistance against *M. incognita* have been widely planted in the southern U.S.A. for 25-50 years, respectively. The effect of widespread or occasional use of these resistant cultivars on reproduction and virulence of field populations of *M. incognita* is unknown.

There has been little research on the damage caused by *M. incognita* to grain sorghum in the mid-South where populations of the nematode reproduce on susceptible cultivars. Additionally, there is not much information available on the host status of current cultivars. There are a few nematicides available for nematode management in sorghum including in-furrow and seed treatments, but crop rotation is still one of the most cost-effective and recommended methods of management used in the U.S.A. (Koenning *et al*., 1999).

Grain sorghum is a relatively minor crop in Louisiana with a total production of 39,000 ha and a value of $37 million in 2014 (Anonymous, 2015a). However, sorghum is widely grown as a rotation crop with cotton, corn, or soybeans and can impact populations of *M. incognita* in production fields. In Louisiana, many of the fields where sorghum is grown have mixed populations of predominantly *M. incognita* and *Rotylenchulus reniformis* (Hague and Overstreet, 2002) making the design of a crop rotation system challenging.

The objectives of this research were to: 1) evaluate the host susceptibility of sorghum cultivars currently used in Louisiana to a population of *M. incognita* race 3; 2) determine the impact of different populations of *M. incognita* race 3 from Louisiana on three cultivars of grain sorghum that vary in susceptibility; and 3) perform molecular characterization of 18S and 28S regions of ribosomal DNA of each nematode population.

MATERIALS AND METHODS

General procedures

Experiments were conducted in a greenhouse environment in 2014 and 2015 to assess the host suitability of currently grown cultivars of grain sorghum to *M. incognita* race 3. Terra cotta pots with a top diameter of 15.0 cm and soil-capacity 1.6 kg were utilized. Soil mixtures used in greenhouse studies were three parts sterilized sandy loam soil and one part sand for a final mixture of 85% sand, 13% silt, and 2% clay. All materials, including soil, were autoclaved for 8 h at 116°C prior to use. Each experiment was repeated once.

Nematode inoculum consisted of nematode eggs extracted from fresh root tissue by shaking in 0.6% solution of sodium hypochlorite (NaOCl) for 5 min (Hussey and Barker, 1973). Air temperature was monitored daily with a HOBOware data logger (HOBO UX100 temperature data logger - UX100-001). There were four replications of each treatment and pots were arranged as a randomized complete block design. Plants were fertilized every 2 wk with water-soluble Miracle-Gro® fertilizer (18% nitrogen, 18% available phosphate, and 21% soluble potash). Aqueous suspensions of 2,000 root-knot nematode eggs were applied into 5-cm deep depressions of soil in pots containing 1-wk-old plants.

At the conclusion of all experiments, 250 cm^3 soil samples were processed by semiautomatic elutriation (Byrd *et al*., 1976), and sugar flotation-centrifugation (Jenkins, 1964). Immature stages of *M. incognita* were enumerated at 40X using an inverted microscope. Eggs were extracted from 5 g of fresh root tissue as previously described and counted using an inverted microscope.

Populations of southern root-knot nematode

Populations of root-knot nematode used in this study were collected in 2013 from cotton, soybean, and sweet potato fields that had plants exhibiting galling symptoms. Soil from each of these locations was added to a 20.3 cm terra cotta pot and a single tomato seedling (*Solanum lycopersicum* L.) cv. 'Rutgers' was transplanted to the center of the pot. After 6 wk, a single egg mass of root-knot nematode was collected from each location and transferred to additional pots with previously established tomatoes. Cultures of root-knot nematode were established representing each location and maintained in the greenhouse on 'Rutgers' tomato. These root-knot single egg mass populations were carefully handled and maintained in the greenhouse to reduce cross contamination.

A modified host differential test (Eisenback and Triantaphyllou, 1991) using 'Rutgers' tomato, 'Deltapine 0912 B2RF' cotton, and 'NC 95' tobacco (data not shown) was conducted with all the nematode populations to assess their host-race status.

Samples of each nematode population were submitted to the Nematode Assay Laboratory of the Agronomic Division at North Carolina Department of Agriculture & Consumer Services (NC-DACS), where species identification was done by species-specific primers, and molecular characterization was performed by sequencing the 18S and 28S regions of ribosomal DNA (rDNA). DNA extraction, PCR amplification, sequencing, and sequence editing followed that of Ye *et al*. (2015). Species-specific primers used for PCR were inc-K14-F (5'GGGATGTGTAAATGCTCCTG3') and inc-K14-R (5'CCCGCTACACCCTCAACTTC3'), which amplify a band of 399 bp for *M. incognita* isolates (Randig *et al*., 2002). DNA sequences generated in this study were compared with nematode sequences from the GenBank sequence database using the blastn algorithm for preliminary identification. Sequences generated for this study were placed into a broader evolutionary context by inferring phylogenetic relationships between Louisiana populations of *M. incognita* and those from a recent phylogenetic study of *Meloidogyne* (Ye *et al*., 2015) that includes all known species. GenBank accession numbers for each of the sequences generated or included in this study are listed in Table 1. Multiple sequence alignments were estimated using MAFFT v. 7 (Katoh and Standley, 2013) under the G-INS-i iterative refinement method via the online server (http://mafft.cbrc.jp/alignment/software/). Phylogenetic relationships were inferred separately for the 18S and 28S sequence alignments and the 18S-28S concatenated matrix using a maximum likelihood (ML) approach. The ML phylogenies were inferred with GARLI v 2.01 (Zwickl, 2006) using the High Performance computing resources at Louisiana State University (http://hpc.lsu.edu). Each of the phylogenetic analyses were conducted assuming the best-fit model of nucleotide evolution selected by jModelTest

Table 1. Species and single egg mass populations of root-knot nematodes (*Meloidogyne* sp.) used in the present study. Samples in bold correspond to those sequenced in this study.

Root-knot nematode species	Host	Locality (County/ Parish, State)	18S + ITS GenBank acession no.	28S D2/ D3 GenBank acession no.	Tip name
M. marylandi	Bermuda grass	USA	KP901041	KP901066	Mmarylandi1
M. marylandi	Bent grass	USA	KP901042	KP901066	Mmarylandi2
M. marylandi	Bermuda grass	Lexington, SC	KP901043	KP901066	Mmarylandi3
M. graminis	Bermuda grass	Kershaw, SC	KJ934143	KP901067	Mgraminis1
M. naasi	Bent grass	Avery, NC	KJ934133	KP901068	Mnaasi1
M. naasi	Bent grass	Avery, NC	KJ934132	KP901069	Mnaasi2
M. incognita	Zoysia grass	USA	KP901045	KP901070	Mincognita1
M. incognita	Bermuda grass	USA	KP901046	KP901072	Mincognita2
M. naasi	Turf grass	USA	KP901048	KP901073	Mnaasi3
M. marylandi	Bermuda grass	USA	KP901049	KP901066	Mmarylandi4
M. graminis	Turf grass	USA	KP901050	KP901074	Mgraminis2
M. graminis	Bermuda grass	USA	KP901051	KP901075	Mgraminis3
M. graminis	Bent grass	USA	KP901054	KP901067	Mgraminis4
M. graminis	Zoysia grass	USA	KP901055	KP901067	Mgraminis5
M. graminis	Fescue	USA	KP901056	KP901077	Mgraminis6
M. incognita	Peach	USA	KP901057	KP901078	Mincognita3
M. enterolobii	Soybean	USA	KP901058	KP901079	Menterolobii
M. chitwoodi	Potato	USA	KP901059	KP901080	Mchitwoodi
M. incognita	Tobacco	USA	KP901060	KP901081	Mincognita4
M. arenaria	Unknown	China	KP901061	KP901082	Marenaria
M. javanica	Unknown	China	KP901062	KP901083	Mjavanica1
M. javanica	Unknown	USA	KP901063	KP901084	Mjavanica2
M. incognita	Unknown	USA	KP901064	KP901085	Mincognita5
M. hapla	Unknown	USA	KP901065	KP901086	Mhapla
M. incognita	**Feathery coxcomb**	**East Baton Rouge, LA**	**MF177711**	**MF177877**	**LARK2**
M. incognita	**Cotton**	**Tensas, LA**	**MF177712**	**MF177878**	**LARK3**
M. incognita	**Soybean**	**Morehouse, LA**	**MF177713**		**LARK10**
M. incognita	**Cotton**	**Concordia, LA**	**MF177714**	**MF177879**	**LARK12**
M. incognita	**Cotton**	**Ouachita, LA**	**MF177715**		**LARK13**
M. incognita	**Cotton**	**Red River, LA**	**MF177716**	**MF177880**	**LARK15**
M. incognita	**Soybean**	**Pointe Coupee, LA**	**MF177717**		**LARK16**
M. incognita	**Cotton**	**East Carroll, LA**	**MF177718**	**MF177881**	**LARK18**
M. incognita	**Sweet potato**	**St. Landry, LA**	**MF177719**	**MF177882**	**LARK19**

v 2.1.6 (Darriba *et al*., 2012). The analysis of the concatenated data was done applying a separate model of nucleotide evolution to each portion of the dataset partitioned by gene region (18S and 28S). The ML estimate of the phylogeny was that which had the highest likelihood after 100 replicate searches and was found more than once across replicates. Nonparametric bootstrapping was used to assess branch support with 1,008 bootstrapped pseudoreplicate datasets with the best tree from two search replicates retained per bootstrap replicate. Bootstrap support values were mapped using the DendroPy phylogenetic computing library v 4.0.3 (Sukumaran and Holder, 2010).

Experiment 1: Sorghum cultivars trial

The objective of this experiment was to evaluate reproduction of *M. incognita* on 29 cultivars of sorghum currently available for the southern U.S.A. This 60-d-duration experiment employed 29 cultivars of grain sorghum (from Chromatin, Inc., Crop Protection Services, DuPont Pioneer, Monsanto and Terral Seed, Inc.), Rutgers VF tomato, and one population of *M. incognita* from a cotton field on the Northeast Research Station in St. Joseph, LA. One-week-old seedlings were inoculated with 2,000 eggs of *M. incognita*. Juveniles, eggs, and gall rating data were collected at the conclusion of the experiment. Gall ratings were based on Bridge and Paige (1980) using a scale of 0 to 10.

Over the course of these experiments, the average temperature in the greenhouse was 29.3°C, with maximum and minimum of 39.1°C and 22.0°C, respectively.

Experiment 2: Sorghum cultivars X M. incognita populations

The objective of this experiment was to evaluate the interaction effect of nematode population and sorghum cultivar on the nematode reproduction. The duration of the second experiment was 80 d and employed three cultivars of sorghum selected according to data from experiment one. The cultivar DEKALB DKS 53-67 was selected as the cultivar supporting the greatest nematode reproduction, REV RV9924 supporting moderate reproduction, and REV RV9782 supports the least reproduction. These three cultivars are among the five highest yielding cultivars planted in Louisiana in 2014 (Mascagni *et al*., 2014). Additionally, ten populations of root-knot nematode from nine Louisiana parishes were utilized.

The average temperature in the greenhouse over the course of this experiment was 29.0°C, with maximum and minimum of 40.9°C and 22.8°C, respectively.

Analysis of data

Data were examined by analysis of variance for a randomized complete block or for a 3 X 10 (sorghum cultivar X nematode population) factorial design. Means were separated with Fisher's Least Significant Difference ($P \leq 0.05$) test. Both runs of each experiment were combined.

RESULTS

Experiment 1: Sorghum cultivars trial

In this experiment, nematode reproduction was significantly affected by sorghum cultivar. Final populations of *M. incognita* ranged from 1,239 to 30,440 eggs/g of root, on REV RV9782 and DEKALB DKS 53-67, and from 1,440 to 29,620 juveniles/500 cm^3 of soil on REV RV9782 and Dyna-Gro M75GB39, respectively. The greatest number of eggs was observed with the cultivar DEKALB DKS 53-67, and the lowest was observed with the cultivar REV RV9782 (Table 2).

Gall ratings across the sorghum cultivars averaged from 0 to 2 on Rev RV9782 and NK 7829, respectively. Because of the extremely small size of most galls and low numbers, gall ratings were discontinued after the first experiment.

Experiment 2: Sorghum cultivars X M. incognita populations

Nematode reproduction differed among the three sorghum cultivars tested. Sorghum cultivar and root-knot nematode population significantly impacted both numbers of eggs/g of root and juveniles/500 cm^3 of soil (Table 3). Overall, nematode reproduction was greater on DEKALB DKS 53-67 (Tables 3 and 4) and LARK19 had the highest level of reproduction across the three sorghum cultivars (Table 5). The interaction between sorghum cultivar and nematode population significantly affected the number of eggs of *M. incognita* (Fig.1) but not the number of juveniles. The cultivar DEKALB DKS 53-67 yielded the greatest number of eggs for six out of ten root-knot nematode populations tested. For all nematode populations evaluated, the least nematode reproduction was observed for REV RV9782.

The cultivars DEKALB DKS 53-67 and REV RV9924 had significantly greater numbers of eggs than REV RV9782 for most of the nematode populations tested (Fig.1). The number of eggs of REV RV9782 did not differ significantly among the different root-knot nematode populations. The greatest nematode reproduction was observed with the LARK19 population for sorghum cultivars DEKALB DKS 53-67, and REV RV9924.

Phylogenetic analysis

All 10 nematode populations used in this

Table 2. Root-galling and soil population densities of *Meloidogyne incognita* recovered from 29 sorghum cultivars in a greenhouse environment[y].

Sorghum cultivar	Gall rating[z]	Eggs/g root	Juveniles/500 cm^3 soil
DEKALB DKS 53-67	1.2	30,440 a	25,208 ab
Pioneer 83P17	1.0	24,256 ab	21,540 a-d
Sorghum Partners SPX 3550	0.8	23,803 a-c	13,120 c-g
Pioneer 83P99	1.0	23,713 a-c	15,420 b-g
Pioneer 84P80	1.2	21,119 a-d	19,310 a-e
Sorghum Partners X446	1.4	18,678 a-e	14,280 b-g
Sorghum Partners SP6929	0.8	16,398 b-f	19,100 a-f
Sorghum Partners SP7868	1.4	16,136 b-f	13,310 c-g
Sorghum Partners X445	1.8	15,176 b-g	13,440 c-g
Sorghum Partners X840	1.6	13,831 b-h	8,430 e-h
Sorghum Partners NK7633	1.6	13,830 b-h	6,320 gh
REV® RV9883™	1.0	13,821 b-h	8,240 f-h
Sorghum Partners SPX 3680	1.3	12,754 b-h	13,840 c-g
REV® RV9924™	1.4	12,366 b-h	9,320 e-h
DEKALB DKS 51-01	0.8	11,213 c-h	7,070 gh
REV® RV9562™	0.8	11,103 d-h	7,940 gh
Dyna-Gro M72GW14	1.6	10,038 d-h	15,120 b-g
Dyna-Gro M77GB52	1.0	9,423 d-h	16,270 b-g
Sorghum Partners X715	1.4	8,898 d-h	7,460 gh
Dyna-Gro 765B	1.6	8,736 d-h	23,180 a-c
Dyna-Gro M77GR61	1.0	8,444 e-h	10,480 e-h
Dyna-Gro M75GB39	0.6	8,371 e-h	29,620 a
Sorghum Partners NK7829	2.0	5,343 f-h	7,580 gh
Sorghum Partners NK 6638	1.2	4,847 f-h	11,650 d-h
Sorghum Partners X865	1.8	4,773 f-h	11,550 d-h
Sorghum Partners SPX 380	1.0	4,533 f-h	13,840 c-g
Sorghum Partners SPX 3675	0.2	3,316 gh	11,970 d-h
Sorghum Partners K73-J6	0.8	3,076 gh	5,676 gh
REV® RV9782™	0.0	1,239 h	1,440 h

[y]The experiment duration was 60 d, and there were a total of eight replications of each treatment. Data analyzed with ANOVA and LSD test ($P \leq 0.05$). Means followed by the same letter in a column are not significantly different.

[z]Gall ratings were on a scale of 0-10 (0 = no galling, 10 = complete galling) and determined only on the first run of the experiment.

Table 3. Main and interaction effects (*P* values) of sorghum cultivar and *Meloidogyne incognita* population on nematode reproduction in a greenhouse environment[x].

Source	DF	Juveniles	Eggs/g root
Sorghum cultivar[y] (S)	2	≤ 0.01*	≤ 0.01*
Nematode population[z] (RK)	9	0.12	≤ 0.01*
S x RK	18	0.29	≤ 0.01*

[x]Data combined over two 80-d duration experiments with a total of 8 replications. Data was analyzed with ANOVA and LSD (*P* ≤ 0.05).
[y]Sorghum cultivars used in this experiment ranged from very susceptible to very resistant. Cultivars employed were: DEKALB DKS 53-67, REV RV9924, and REV RV978.
[z]Nematode populations were collected from different Louisiana Parishes.
*Indicates a significant *P* value.

Table 4. Population densities across 10 populations of *Meloidogyne incognita* recovered from three sorghum cultivars in a greenhouse environment[y].

Sorghum cultivar	Eggs/g root[z]	Juveniles/500 cm^3 soil[z]
DEKALB DKS 53-67	37,695 a	13,835 a
REV RV9924	27,833 b	8,248 b
REV RV9782	4,059 c	3,176 c

[y]The experimental duration was 80 d and data are the means of 8 replications averaged over two trials.
[z]Data analyzed with ANOVA and LSD test (*P* ≤ 0.05). Means followed by the same letter in a column are not significantly different.

Table 5. Population densities of the 10 populations of *Meloidogyne incognita* race 3 recovered from across three sorghum cultivars in a greenhouse environment[y].

Place of origin of *M. incognita* population (LA Parish)	*M. incognita* population	Eggs/g root[z]	Juveniles/ 500 cm^3 soil[z]
St. Landry	LARK19	53,877 a	13,413 a
East Baton Rouge	LARK2	26,236 b	7,007 bc
Morehouse	LARK10	23,583 b	6,497 bc
Ouachita	LARK13	22,714 b	9,463 ab
Red River	LARK15	20,464 b	6,470 bc
Pointe Coupee	LARK16	19,619 b	9,967 ab
East Carroll	LARK18	18,705 b	8,340 abc
Concordia	LARK12	17,571 b	9,590 ab
Tensas	LARK3	16,913 b	9,760 ab
Morehouse	LARK6	12,275 b	3,690 c

[y]The experimental duration was 80 d, and data are the means of 24 replications averaged over two experiments.
[z]Data analyzed with ANOVA and LSD test (*P* ≤ 0.05). Means followed by the same letter in a column are not significantly different.

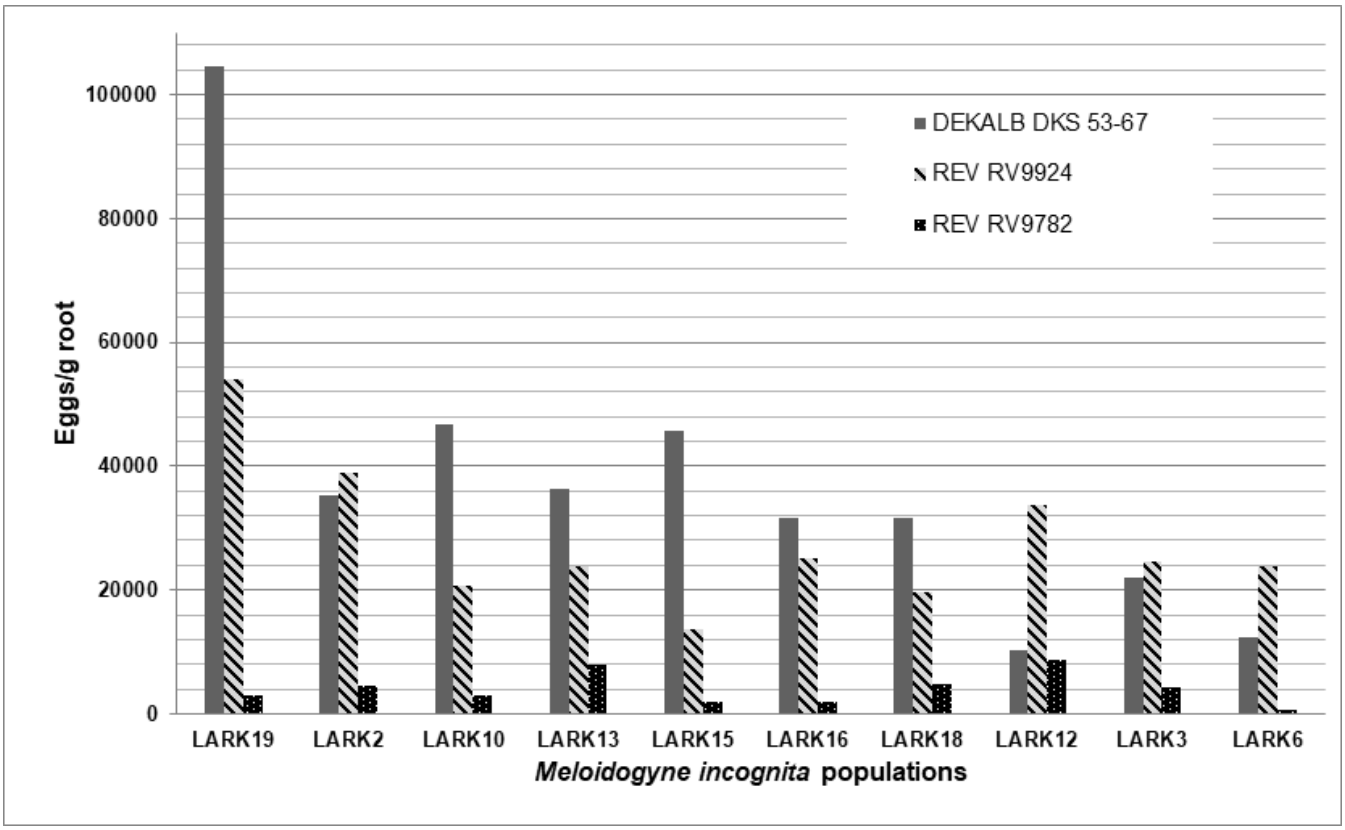

Fig. 1. Individual treatment means for the interaction between sorghum cultivar and root-knot population and effect on egg production on three sorghum cultivars in a greenhouse environment. Data are means of eight replications averaged over two trials. Across all columns, means followed by the same letter do not differ significantly according to LSD test, $P \leq 0.05$.

study were confirmed to be *M. incognita* based on phylogenetic analyses of 18S and 28S rDNA. The independent gene trees inferred from 18S and 28S were congruent with one another and with the phylogeny inferred from the concatenated dataset (Fig. 2).

Cotton and tomato were extensively galled by all root-knot nematode populations in the host differential test, while tobacco had no detectable galling from any population, which indicates that the root-knot was *M. incognita* race 3. Results from the molecular characterization performed by NC-DACS confirmed that all populations were *M. incognita* (Fig. 3). The presence of a band of about 400 bp with inc-K14-F/inc-K14-R primers in the populations tested reaffirms the reliability of the species identification. There was no amplification with the negative control.

DISCUSSION

Results from the greenhouse studies reveal that the current sorghum cultivars being evaluated in Louisiana varied considerably in their reaction to *M. incognita*. The 29 cultivars evaluated ranged from very susceptible to moderately resistant to the nematode. These results confirm the variability found in the susceptibility of sorghum to *M. incognita* previously reported (Birchfield, 1983; Babatola and Idowu, 1990; McSorley and Gallaher, 1992; Koenning *et al*., 1999; McClure *et al*., 1999; Davis *et al*., 2015).

Birchfield (1983) found that 8 of 9 sorghum cultivars evaluated in Louisiana were susceptible and one cultivar was moderately resistant based on galling by the nematode, which averaged from 81 to 100% for 78% of the varieties tested. The gall ratings in the current study were lower, and the averages were never higher than 2.0 according to Bridge and Page (1980) scale of 0-10. The galls observed in this study were very small and generally difficult to detect without magnification. Our results were based primarily on egg production and numbers of juveniles found in the soil. However, the results indicated that 28 of the 29 sorghum cultivars evaluated would be rated as susceptible and one as moderately resistant.

Fig. 2. Maximum likelihood phylogeny of *Meloidogyne* spp. based on a concatenated matrix of 18S and 28S sequences. The tree is rooted at the midpoint of the path between the two most distant taxa on the tree. Bootstrap support values >70 are shown above the branches. The scale bar represents the estimated number of substitutions per site.

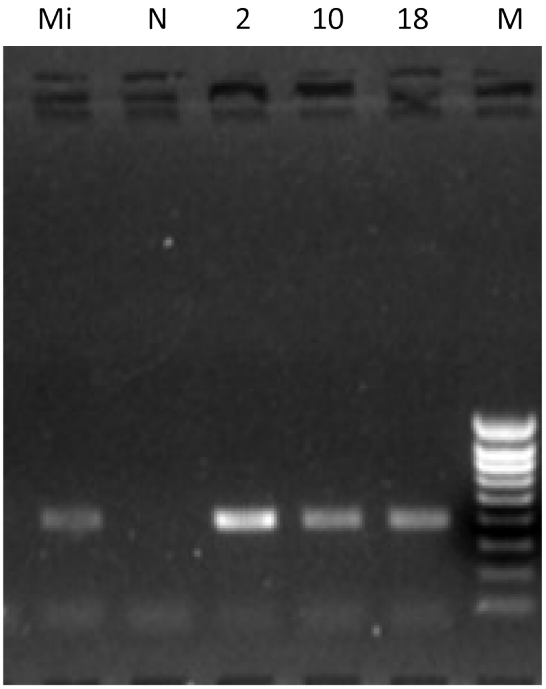

Fig. 3. PCR amplification product generated using species-specific primers: inc-K14-F/inc-K14-R (399 bp). Mi = *Meloidogyne incognita* (positive control), N = negative control, 2 = LARK2, 10 = LARK10, 18 = LARK18, M = 1kb DNA ladder (Genesee Scientific). These results are products from molecular characterization performed by Nematode Assay Laboratory of the Agronomic Division at North Carolina Department of Agriculture & Consumer Services.

The lack of resistance for root-knot nematode in the currently used sorghum cultivars/lines limits the use of this crop in a rotation system in fields where *M. incognita* is a problem. A careful selection of sorghum cultivar may help to reduce yield losses in sorghum and in the next crops planted in a rotation system. In 1991, Gallaher *et al*. observed a significant reduction in *M. incognita* populations after planting sorghum in a corn and sorghum cropping system. McSorley and Gallaher (1993) have also reported decreases in *M. incognita* populations after sorghum. Harris-Shultz *et al*. (2015) evaluated crosses of resistant and susceptible cultivars of sweet sorghum and developed markers for moving the resistance gene for *M. incognita* to any sorghum line.

In these current studies, the greatest variability among populations of *M. incognita* was observed in the most susceptible and moderately susceptible sorghum cultivars, but not in the more resistant cultivar. The use of less susceptible cultivars could help decrease nematode populations below threshold levels. The low egg counts observed for the sorghum cultivar REV RV9782 for all the 10 populations of *M. incognita* tested indicates that this cultivar could be a good choice for use in a rotation system where this nematode is present. Therefore, the characterization of host susceptibility of grain sorghum to *M. incognita* within geographical areas could greatly contribute to the use of this crop for management of the southern root-knot nematode.

Identification of *Meloidogyne* species by analysis of DNA sequences has been routinely used in recent years (McClure *et al*., 2012). In this study, intraspecific variation was not detected among 18S and 28S sequences from 10 populations of *M. incognita*, despite variability in reproduction rate across a broad geographical distribution. For example, the populations LARK2 and LARK6 had identical 18S and 28S haplotypes despite a geographical separation of 305 km and a more than two-fold difference in the number of eggs produced per gram of root. However, the lack of variation in rDNA may not be representative of genome-wide genetic variation due to nucleotide conservation across *Meloidogyne* lineages. Further genetic characterization is warranted to detect variation among the sampled populations.

The phylogenetic tree based on 18S and 28S showed *M. incognita* and *M. javanica* nested within the same clade when compared to sequences representing several other species of *Meloidogyne*. Previous studies have reported a similar relationship between these two species based on phylogenetic analyses of the 18S rRNA region and the internal transcribed spacer (ITS) (McClure *et al*., 2012) or reproduction systems (Holterman *et al*., 2009). Studies with mitochondrial DNA (mtDNA) have also reported great similarities among *M. incognita* and *M. javanica* due to their similarity in PCR products (Powers and Harris, 1993; Jeyaprakash *et al*., 2006; Baidoo *et al*., 2016).

The results of this study indicate that the variability of sorghum susceptibility to the different populations of *M. incognita* cannot be attributed to the geographical location or rDNA sequence of the nematode. Further studies are necessary to unveil the unknown aspects of the variability in host-pathogen interaction for better management of this pathogen.

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