FIRST REPORT OF *MELOIDOGYNE PARTITYLA* PARASITIZING PECAN IN ARKANSAS AND CONFIRMATION OF *QUERCUS STELLATE* AS A HOST

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ABSTRACT

Khanal, C., A. L. Szalanski, and R. T. Robbins. 2016. First report of *Meloidogyne partityla* parasitizing pecan in Arkansas and confirmation of *Quercus stellate* as a host. Nematropica 46:1-7.

Pecan (*Carya illinoinensis*) is a valuable tree-nut and lumber crop in the USA. Pecan orchards of Georgia, New Mexico, Florida, Texas, Arizona, and Oklahoma have been reported to be infested by the root-knot nematode, *Meloidogyne partityla*. A survey was conducted from several pecan orchards in Arkansas to look for *M. partityla*. Soil and root samples were collected from one established pecan orchard in each of six different counties in the state. Pecan roots showing galling, and second-stage juveniles (J2) of a *Meloidogyne* species were detected in a Logan County orchard. Molecular and morphometric studies using both J2 and females confirmed the presence of *M. partityla*. This is the first report of *M. partityla* in Arkansas. Additionally, greenhouse studies with *Quercus stellate* confirmed post oak as a suitable host of *M. partityla*.

Key Words: Arkansas, *Carya illinoinensis*, *Meloidogyne partityla*, *Quercus stellate*.

RESUMEN

Khanal, C., A. L. Szalanski, and R. T. Robbins. 2016. Primira cita de *Meloidogyne partityla* parasitando pacana en Arkansas y confirmación de *Quercus stellate* como hospedante. Nematropica 46:1-7.

La pacana (*Carya illinoinensis*) es un importante cultivo productor de nueces y madera en USA. Se han citado infestaciones por el nematodo formador de agallas en las raíces, *Meloidogyne partityla* , en huertos de pacana de Georgia, New Mexico, Florida, Texas, Arizona, y Oklahoma. Se llevó a cabo una prospección para buscar *M. partityla* en varios huertos de pacana en Arkansas. Se recolectaron muestras de suelo y raíces en un huerto de pacana de cada unos de los diferentes condados del estado. Se detectaron raíces de pacana mostrando agallamiento y juveniles de segundo estadio (J2) de una especie de *Meloidogyne* en un huerto del condado de Logan. Estudios moleculares y morfológicos de J2 y hembras confirmaron la presencia de *M. partityla*. Esta es la primera cita de *M. partityla* en Arkansas. Adicionalmente, estudios en invernadero con *Quercus stellate* confirmaron al roble de los postes como un hospedante adecuado para *M. partityla*.

Palabras clave: Arkansas, *Carya illinoinensis*, *Meloidogyne partityla*, *Quercus stellate.*

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is a valuable tree-nut and lumber crop in the family Juglandaceae along with walnut and hickory. Thought to be native to the United States, pecan is found from Texas in the southern United States to Iowa in the north (Sparks, 1991). Of 14 pecan-producing states, Georgia is the leading state in terms of production followed by New Mexico and Texas (Brito *et al*., 2006). Pecan production in Arkansas in 2014 was estimated to be 1,588 metric tons (United States Department of Agriculture, 2015).

Kleynhans (1986) reported a new *Meloidogyne* species, (*Meloidogyne partityla* Kleynhans, 1986),

the pecan root-knot nematode, from South Africa. This root-knot species was possibly introduced into South Africa during import of pecan seedlings from the United States (Kleynhans, 1986). Since then, this species has been found in other parts of the world including in the United States in Texas (Starr *et al*., 1996), and Georgia, New Mexico, Florida, Arizona, and Oklahoma (Brito *et al.*, 2006) posing a threat to the pecan industry. The nematode has been reported to attack hickory (*Carya ovata*), walnut (*Juglans hindsii* and *J. regia*), and pecan (Starr *et al.*, 1996). In addition, Brito *et al.* (2013) described laurel oak (*Quercus laurifolia* Michx) as a host of this nematode. The presence of *M. partityla* in Arkansas before this study was unknown.

Sample collection and nematode extraction

A survey of several pecan orchards in Arkansas was conducted with an interest to evaluate the presence of *M. partityla* in this state. Soil samples from a pecan orchard in Pope (near Russelville), Logan (near the Arkansas River), Faulkner, Conway, Craighead, and Miller Counties were taken. Pecan orchards ranged in size from 0.2 to 5 ha. Samples were taken from 3 to 8 pecan trees representative of the orchards, primarily from the ones showing stress or decline symptoms. Attempts were made to collect pecan roots from each tree sampled. In an attempt to avoid grass roots, the upper 10 cm of soil was removed using a shovel and approximately 3 kg soil was collected to a depth of 10 to 20 cm. A single tree constituted a single sample. Samples from each tree were kept in plastic bags, labelled, and processed separately. To evaluate the presence of second-stage juveniles (J2) of *Meloidogyne* spp., the soil samples were processed using a rapid centrifugal-flotation technique as described by Jenkins (1964). Roots were examined under compound microscope at 40× to determine the presence of galls.

Of the six pecan orchards sampled, root-knot nematodes were found in only one of the samples from Logan County. Pecan roots with distinct galls (Fig. 1) were found when examined under stereoscopic microscope. Approximately 20 J2 (Fig. 2) per kg soil were obtained. No males were found in soil or root samples. Females were excised from root galls (Fig. 3) using a sterilized needle, scalpel, and a stereoscopic microscope for DNA extraction. The other samples collected were found to be negative for the presence of root-knot nematodes.

Molecular characterization

Using the smash method described by Powers and Harris (1993), DNA from individual J2 from soil was extracted. The suspension containing pieces of nematode served as a template for the Polymerase Chain Reaction (PCR). DNA from individual females that had been dissected from roots was extracted using the puregene technique, a protocol based on rapid isolation of mammalian DNA (Sambrook and Russell, 2001a). Ten individual J2 and three females were processed separately for molecular study. Polymerase chain reaction was performed using the DNA template obtained from individual J2 or females. The primers used were C2F3 (5'GGTCAATGTTCAGAAATTTGTGG3') and 1108 (5'TACCTTTGACCAATCACGCT3'), which amplify the region between COII and 16S ribosomal mitochondrial genes (Powers and Harris, 1993; Powers *et al.*, 2005).

The PCR reaction master mix was prepared in such a way that each reaction received 2.5 µl of 10× CL buffer (Qiagen, mat no. 1032517), 17.5 µl of PCR water, 1 µl of 25 mM MgCl2 19 (Qiagen,

Fig. 1. Pecan root with galls and egg masses of *Meloidogyne partityla*.

Fig. 2. *Meloidogyne partityla* second-stage juvenile obtained from pecan orchard in Arkansas.

mat no. 1005482), 0.5 µl of dNTP (10 mM each) (Qiagen, mat no. 1005631), 0.5 µl of 10 µM of each primer (Operon), and 0.25 µl Taq DNA polymerase (5 units/µl) (Qiagen, mat no. 1005476). To the PCR reaction master mix, 2.5 µl of DNA from a J2 or a female described above was added and mixed. Polymerase Chain Reaction (PCR) was performed in a PTC-100® Peltier Thermal Cycler. Amplification conditions included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 45 s, and extension at 72°C for 90 s. A final extension step was conducted for 10 min at 72°C.

Agarose gel (2%) was prepared by mixing 2 g agarose in 100 ml 1× TBE solution and staining with 3.5 µl of Gel Red Nucleic Acid (Biotium, cat: 41003). A 5 µl of 100 bp DNA ladder (Promega, ref: G210A) was loaded in one well while 5 µl samples from the PCR product were loaded in other wells. Agarose gel was run at 168V for 50 min. Separated DNA bands were visualized using a UV transilluminator (UVP BioDoc-ItTM System) (Fig. 4).

Polymerase Chain Reaction product that remained after running a gel was purified using ethanol precipitation of DNA in microfuge tubes (Sambrook and Russell, 2001b). The purified PCR products were prepared according to sample preparation guidelines provided by the DNA Resource Center at the University of Arkansas, Fayetteville, AR. DNA samples were sequenced in both directions by the DNA Resource Center (University of Arkansas, Fayetteville, AR) using an ABI 3130xl analyzer BigDye 3.1 chemistry to provide automated DNA sequencing. Of the 13 PCR samples submitted, sequencing results were obtained only from eight: five from J2 and three from females. Pairwise alignment of forward and reverse sequences was performed using ClustalW (Thompson *et al.*, 1994) to get consensus sequences. Consensus sequences obtained were compared with non-redundant sequences available in GenBank through Nucleotide BLAST (Basic Local Alignment Search Tool). Nucleotide BLAST results confirmed the root-knot species in Logan County to be *M. partityla*. Intraspecific variation was not observed among the specimens sequenced. The DNA sequence of *M. partityla* from the current study was submitted to GenBank (accession number KP975420). Sequence identity of *M. partityla* from Arkansas was 99% with GenBank sequence AY672412 and 95% with GenBank sequence AY672413.

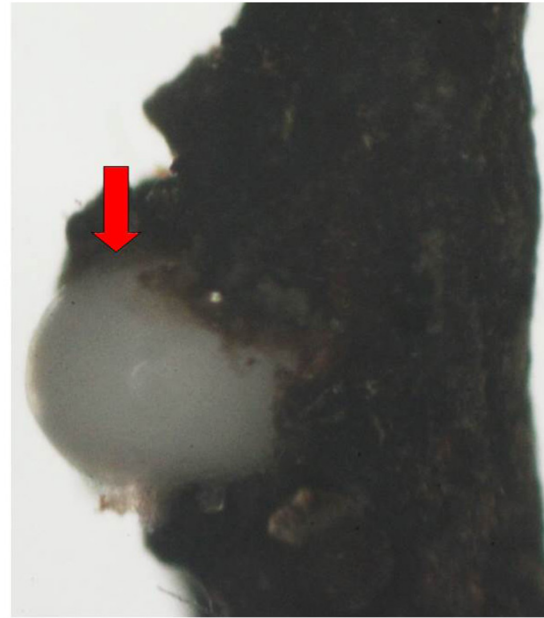

Fig. 3. *Meloidogyne partityla* female protruding from post oak root.

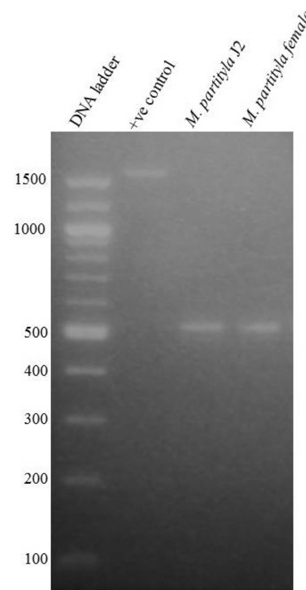

Fig. 4. Visualization of PCR amplicons in agarose gel. *Meloidogyne incognita* was used as positive control.

Morphological characterization

A morphometric study was conducted for the root-knot juveniles, eggs, and mature females. For this, females with an egg mass were excised from pecan root galls using scalpel, needle, and stereoscopic microscope. Egg masses were kept in a petri plate at room temperature for a week to allow J2 to hatch. Hatched J2 and females were prepared according to guidelines provided by Seinhorst (1962). Measurements were taken from 25 J2, 25 eggs, and 10 females. For J2, body length, anal body width, distance from stylet base to head end, distance from stylet base to DGO, distance

from head end to metacorpus valve, distance from head end to excretory pore, tail length, and hyaline tail terminus length (Table 1) were in the range of the original description of *M. partityla* (Kleynhans, 1986). Second-stage juveniles from current study were found to have a narrower body (body width 15.2 ± 1.4 µm) than the original description (17.1 ± 1.3 µm). Similarly, stylet length of the J2 was comparatively longer (13.2 ± 1 µm) than the original description (11.4 ± 0.5 µm). Length and width of eggs from the current study were in a range of the original description (Table 2). For females, body length, neck length, stylet length, stylet cone length, vulva length, and distance from vulva to anus were within the range of the original description (Table 3). Female body width suggests that the females from this study were more obese (505.3 ± 93.5 µm) than the original description (462 ± 67.6 µm). Overall, morphometric analysis of J2, eggs, and females shows that *M. partityla* from Arkansas shares similar morphometry with the original description by Kleynhans (1986).

Molecular phylogenetic relationships

Phylogenetic analysis of the mitochondrial gene was performed to examine the relationships of *M. partityla* from Arkansas with other *Meloidogyne* sequences available in Genbank. Sequences were aligned using Geneious software version 6.1.8 (Kearse *et al.*, 2012). Bayesian tree was constructed with the best-fitting nucleotide substitution model chosen in accordance with the general time reversible + gamma (GTR+G) model among 64 different models using the Model Test v 3.7 (Posada and Crandall, 1998) and PAUP 4.0b10 (Swofford, 2001) programs. Phylogenetic trees were obtained using Bayesian inference with the GTR+G model using BEAUti and the BEAST 1.7 software (Drummond *et al*., 2012). *Globodera rostochiensis* (GenBank accession number EF462977) was used as an outgroup (Fig. 5). Bayesian analysis shows the Arkansas *M. partityla* falls in the same clade as other *M. partityla* sequences available in GenBank. The tree suggests that some variation exists

Table 1. Measurements of second-stage juveniles (n = 25) of *Meloidogyne partityla* from Arkansas and comparison with the original data from Kleynhans (1986). Measurements are in the format of mean ± standard deviation (ranges).

Parameters	Current study (µm)	Kleynhans, 1986 (µm)
Body length	447.5 ± 32.2 (387.3-486.2)	437 ± 25.3 (383-494)
Body width	15.2 ± 1.4 (10.2-16.7)	17.1 ± 1.3 (14.7-19.6)
Anal body width	11.1 ± 1 (10.2-13.2)	11.7 ± 0.7 (10.7-13.2)
Stylet length	13.2 ± 1 (10.7-15.2)	11.4 ± 0.5 (10.3-12.4)
Stylet base to head end	14.1 ± 0.7 (13.1-15.7)	15.8 ± 0.7 (14.1-17.5)
Stylet base to DGO	3.8 ± 0.7 (2.5-5.6)	2.9 ± 0.4 (1.7-3.7)
Head end to metacorpus valve	62.3 ± 1.9 (58.4-66.9)	60.5 ± 2.7 (55.4-67.1)
Head end to excretory pore	86.8 ± 4.71 (79.2-97.4)	87.4 ± 4.8 (75.3-95.4)
Tail length	51.9 ± 4.1 (44.7-58.9)	50.5 ± 4.9 (45-63.5)
Head end to esphagus end (B2)	132 ± 7.3 (121.8-150.2)	Not available
Hyaline tail terminus length	15.1 ± 2.7 (11.7-18.3)	14 ± 1.7 (11.4-18.8)

Table 2. Measurements of eggs (n = 25) of *Meloidogyne partityla* from Arkansas and comparison with the original data from Kleynhans (1986). Measurements are in the format of mean ± standard deviation (ranges).

Parameters	Current study (μm)	Kleynhans, 1986 (μm)
Length	100.2 ± 8.5 (85.3-114.7)	99.8 ± 8 (82-116)
Width	44.1 ± 3.8 (38.6-49.7)	43.7 ± 3.3 (36.7-49.2)

Table 3. Measurements of females (n = 10) of *Meloidogyne partityla* from Arkansas and comparison with the original data from Kleynhans (1986). Measurements are in the format of mean ± standard deviation (ranges).

Parameters	Current study (μm)	Kleynhans, 1986 (μm)
Body length	819 ± 150 (552.1-995)	812 ± 93.6 (616-993)
Body width	505.3 ± 93.5 (352.4-642.7)	462 ± 67.6 (331-596)
Neck length	210.9 ± 69 (103.7-373.1)	Not available
Stylet length	17.2 ± 2.7 (14.2-19.3)	16.6 ± 1.2 (14.6-20)
Stylet cone length	10.32 ± 1.69 (7.1-11.2)	9.1 ± 0.9 (7.7-11.2)
Vulva length	23.65 ± 3.48 (18.3-28.4)	23.0 ± 2.0 (19.3-27.5)
Distance vulva to anus	17.57 ± 4.12 (12.2-24.4)	18.2 ± 1.8 (16-23.7)

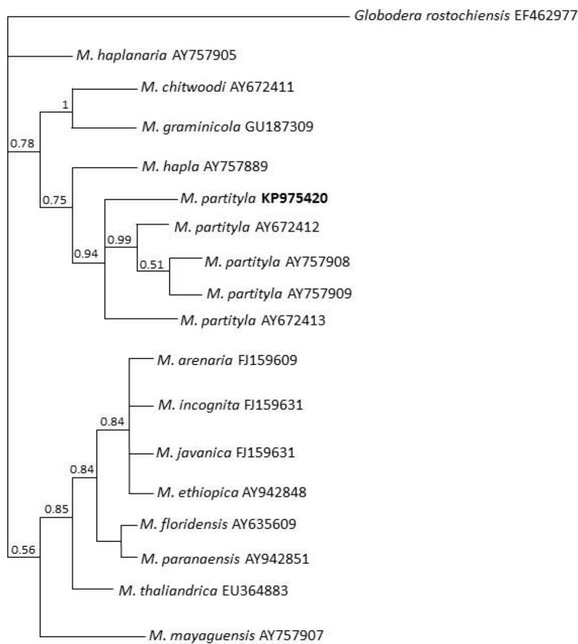

Fig. 5. Bayesian tree inferred from mitochondrial DNA sequence under GTR+I+G model (-lNL = 2438.149; freqA = 0.3074; freqC = 0.0659; freqG = 0.1252; freqT = 0.5014; R(a) = 1.27417; R(b) = 3.35613; R(c) = 1.37264; R(d) = 1.16204; R(e) = 1.32528; R(f) = 1. Numbers at clade branchpoints represent posterior probability values. *Meloidogyne partityla* with GenBank accession number from current study is shown in bold letters.

between *M. partityla* sequences that are available in Genbank.

Inoculation of post oak (Quercus stellate Wangenh.)

Because laurel oak (*Quercus laurifolia* Michx) was reported as a host of *M. partityla* (Brito *et al*., 2013), post oak (*Quercus stellate*) was evaluated as a host by planting seedlings into soil that was positive for the presence of root-knot nematodes. Seedlings were planted in 15-cm-diam. clay pots, allowed to grow in a greenhouse at ambient temperature of 27 ± 5°C, and watered twice a day. Pecan seedlings were also simultaneously planted into the root-knot positive soil. Root galls were detected in both post oak and pecan roots 90 d after inoculation. Females and egg masses were dissected from the roots and identified as *M. partityla* using PCR and gel electrophoresis method as described earlier. This result confirmed post oak as a new host for *M. partityla*. Because post oak belongs to the family Fagaceae, evaluation of other plants in this family should be examined as the possible hosts for *M. partityla*. Our results also suggest that care should be taken in establishing pecan orchards in areas where post oaks are endemic.

This is a first report of *M. partityla* in Arkansas. Pecan trees infected with this nematode had a few small galls on roots, and some of the upper branches appeared to be dying or had small leaves suggesting stress. The relatively low density of root-knot nematodes recovered from the sample is indicative of their possible recent introduction into the orchard. The potential importance of *M. partityla* in Arkansas cannot be determined based on this limited survey, but its presence implies that it is resident in the state. Further studies are needed to determine its range of distribution in pecan orchards in Arkansas.

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