MELOIDOGYNE SPP. IDENTIFICATION AND DISTRIBUTION IN ALABAMA CROPS VIA THE DIFFERENTIAL-HOST TEST AND MOLECULAR ANALYSIS

W. Groover
K. S. Lawrence
Auburn University
Auburn, Alabama

Abstract

The root-knot nematode (*Meloidogyne* spp., RKN) is one of the most commonly found and pathogenic plant-parasitic nematodes on cotton in the United States. Two different techniques were performed to determine their efficacy for *Meloidogyne* species identification. These techniques were the differential-host test (DHT) and molecular analysis. These were initially used on a known greenhouse population of *Meloidogyne incognita* race 3 that had previously been identified by the differential-host test. Initial results showed a confirmation of species with the differential-host test and PCR amplification. Statewide collection of RKN species in Alabama yielded 75 samples from grower's fields in 14 counties. Molecular analysis (PCR) and the differential-host test were used for species identification. Primer sets IncK-14F/IncK-14R (*M. incognita* specific) and Far/Rar (*M. arenaria* specific) were the most robust primer sets used, and were able to identify all 75 samples to species level. Of these samples, 73 were identified as *M. incognita* (97%), and two were identified as *M. arenaria* (3%). Overall, *M. incognita* is the most prevalent species of RKN found on cropping systems in Alabama during this project.

Introduction

Root-knot nematodes (*Meloidogyne spp.*) cause significant yield losses in almost all crop varieties grown in the southeastern United States. As one of the most commonly found plant-parasitic nematodes throughout the world, it is estimated to account for approximately 14% of crop yield loss per year. This accounts for an estimated 100 billion dollars per year (Mitkowski and Abawi 2003). In the state of Alabama alone, around 2.0% of total cotton yield was lost during the 2016 season, which comes out to around 14,200 bales total (Lawrence et al. 2016). Once a field has become infested with these nematodes, a management strategy must be implemented to maximize yield. This yield maximization must be financially practical as well. An effective management option that can be financially viable for containment of root-knot nematode population density is the implementation of a crop rotation. By knowing the specific species of *Meloidogyne* present in the field, a grower can easily implement a crop rotation where the species will not successfully reproduce. For example, if a grower has a cotton field with an infestation identified as *Meloidogyne incognita*, the logical rotation is to peanut next season, since peanut is not a viable host for *M. incognita*. This will lower the nematode population density in the field. Thus, it is important to have quick and efficient ways to identify root-knot nematode species in the field to determine how to manage the infestation.

A common way to identify *Meloidogyne* species is through a differential-host test. This test involves inoculating a population of root-knot nematodes on multiple crops to determine which of the crops will act as a host of the nematode population. Based upon which plants support reproduction of nematodes on the roots, species identification is possible (Barker et al. 1985). However, this test can take 45 days to obtain results. Thus, a more time efficient method is desired. Currently, molecular identification allows for identification of species at a rate of 3 to 4 days (Adam et al. 2006). Thus, DNA analysis is a much more efficient method than the differential-host test.

In this study, the differential-host test along with a molecular identification method using PCR (polymerase chain reaction) and gel electrophoresis with all known primer sets for likely *Meloidogyne* spp. for Alabama were used. The objective of this study was to implement an assay that can quickly and accurately identify root-knot nematode species in Alabama.

Materials and Methods

Differential-host test analysis

Seventeen total populations were analyzed using the differential-host test. Each population was inoculated over 8 different crops: cotton, tobacco, pepper, watermelon, peanut, tomato, corn, and soybean. Corn and soybean are not originally in the DHT, but were included since acreage of these crops is high in Alabama. The DHT was set up as a randomized complete block design with five replications, and RKN eggs were inoculated at a rate of 1000 eggs per 500 cm^3 polystyrene pot. Soil used was a Kalmia loamy sand (80% sand, 10% silt, 10% clay), mixed with sand at a

rate of 60:40 soil to sand. Temperatures in the greenhouse ranged from 24°C to 35°C. After 45 days, root-knot eggs were extracted by shaking the roots in a 10% NaOCl solution for 4 minutes, followed by the collection of eggs on a 25-µm sieve. Eggs were counted and a reproductive factor (Rf) was determined using the following formula: Rf = final egg density ÷ initial egg density. If Rf was over 1, the crop was considered a host, and if less than 1 was considered a nonhost.

PCR amplification and gel electrophoresis of individual nematode samples

PCR was run using individual juveniles that were picked out from extracted samples and placed into 20 µl of water, where they were smashed by a pipette tip and served as DNA template for PCR. Once the sample had been smashed and prepped as the DNA template, it was added to a PCR mix containing 9 µl of DNA template per sample, 20µM of both forward and reverse primers, and 2x JumpStart REDTaq ReadyMix as described by Powers and Harris (1993). Various primer sets were screened to determine which sets are the most efficient and provided the highest percentage of successful amplifications. Both species specific primers as well as primers that differentiate species were screened. The final decision on what primer sets used for the diagnostic assay was determined by how well they perform on a consistent basis. The species specific primer sets were much more reliable compared to the primers that differentiate multiple species, thus the species specific primers were used as described by Adam, Blok, and Phillips (2006). The collected samples were run on PCR via a Labnet MultiGene PCR machine. The amplified products were run on gel electrophoresis by a Labnet Gel XL Ultra V-2 gel reader. At the end of the PCR run, gel electrophoresis was run on a 1% agarose gel to separate the amplified bands, allowing for proper identification of *Meloidogyne* species to be determined.

Results and Discussion

Overall, approximately 100 soil and plant samples thought to have root-knot nematodes were collected. Seventy-five of these samples had RKN present. The differential-host test was run on 17 of these populations of RKN. Of these 17 populations, 15 were successfully identified to species. Thirteen were identified as *M. incognita* race 3, and two were identified as *M. arenaria* race 1. An example of the differential-host test setup can be seen in Figure 1. All 75 RKN samples were identified with species specific primers. Seventy-three were identified as *M. incognita* and two were identified as *M. arenaria.* Figure 2 shows an example of how the species specific primers were used for species identification. Lanes 1 and 2 were identified as *M. incognita* using IncK-14 primers (specific for *M. incognita)*, and lane 3 was identified as *M. arenaria* using Far/Rar primers (specific for *M. arenaria*). All other lanes were run using primers that did not amplify in the presence of those species.

Figure 1. Differential-host test on RKN population 21 days after inoculation.

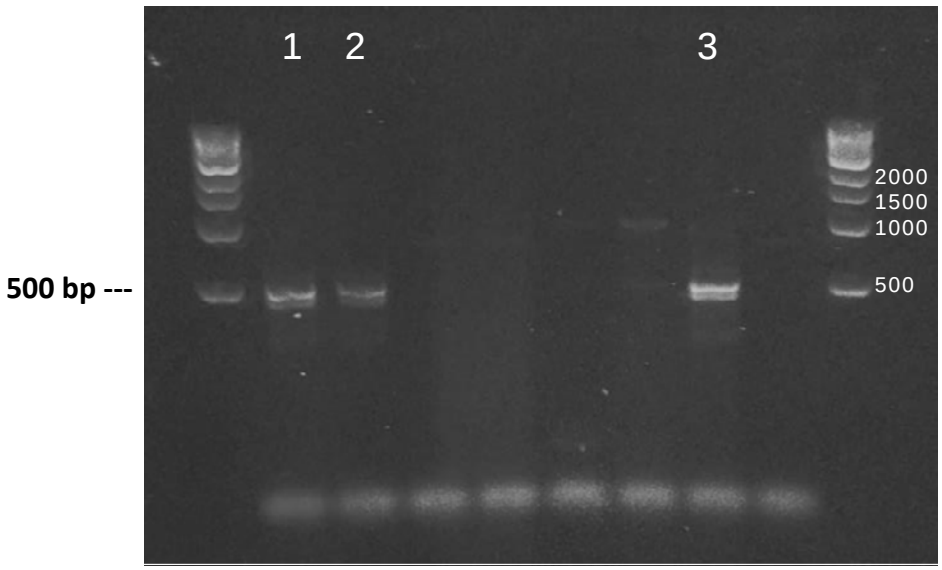

Figure 2. Lanes 1 and 2 showing amplification of *M. incognita* with IncK14 primer set. Lane 3 showing amplification of *M. arenaria* with Far/Rar primer set. Both primers produce a 500 basepair fragment.

Summary

Meloidogyne incognita was by far the most prevalent species found in Alabama, with 73 of the 75 (96%) populations found being identified as *M. incognita*. Two populations were identified as *M. arenaria* (3%). While the creation of a diagnostic tool for species identification has successfully been implemented, the results are somewhat unusual based upon several previous surveys that found a wider variety of species in other regions of the United States. For example, a study in Arkansas identified *M. incognita, M. haplanaria, M. marylandi, M. hapla, M. arenaria* and *M. partityla* (Khanal et. al. 2016). Thus, while only two species have been found to this date in Alabama, there are likely more species present in Alabama.

References

Adam, M., Blok, V., Phillips, M. (2006). Molecular Diagnostic Key for Identification of Single Juveniles of Seven Common and Economically Important Species of Root-Knot Nematode (*Meloidogyne spp.*). Plant Pathology, 56, 190-197.

D'Herde, C. J., Gooris, J. (1972). A Method for the Quantitative Extraction of Eggs and Second Stage Juveniles of *Meloidogyne* spp. From Soil. Ghent, 96, 36.

Khanal, C., Robbins, R.T, Faske, T.R., Szalanski, A.L., McGawley, E.C., Overstreet, C. (2016). Identification and haplotype designation of *Meloidogyne* spp. of Arkansas using molecular diagnostics. Nematropica 46: 261-270.

Lawrence, K., Hagan, A., Norton, R., Faske, T., Hutmacher, R., Muller, J., Wright, D., Small, I., Kemerait, B., Overstreet, C., Price, P., Lawrence, G., Allen, T., Atwell, S., Jones, A., Thomas, S., Goldberg, N., Boman, R., Goodson, J., Young, H., Woodward, J., Mehl, H. (2016). Cotton disease loss estimate committee report, 2016. Proceedings of the 2017 Beltwide Cotton Conference Vol. 1: 188-190. National Cotton Council of America, Memphis, TN. http://www.cotton.org/beltwide/proceedings

Mitkowski, N.A., Abawi, G.S. (2003). Root-knot nematodes. The Plant Health Instructor. DOI:10.1094/PHI-I-2003-0917-0

Powers, T., Harris, T. (1993). A Polymerase Chain Reaction Method for Identification of Five Major *Meloidogyne* Species. Journal of Nematology, 25, 1-6.