# DIFFERENTIATION OF *MELOIDOGYNE* SPECIES WITH FAME ANALYSIS

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## ABSTRACT

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Fatty acid methyl ester (FAME) analysis can be used as a means for differentiating among plant-parasitic nematode genera. The objective of this study was to evaluate the use of this system to identify species and races within a single genus. Fatty acids were extracted and analyzed from samples containing 1000 individuals each of *Meloidogyne arenaria* (race 2), *M. hapla*, *M. incognita* (races 1, 2, and 3), and *M. javanica*. The resulting profiles generated by the Sherlock® Analysis Software were then statistically analyzed with SAS version 9.1.3. The profiles of each *Meloidogyne* species and race were significantly different. The four *Meloidogyne* species separated with a minimum squared Mahalanobis distance ($D^2$) between *M. incognita* and *M. arenaria* ($D^2 = 15.9$, $P < 0.0001$). $D^2$ values among *M. incognita* races were all significant at $P < 0.0001$ with a minimum distance between *M. incognita* race 1 and *M. incognita* race 2 of 17.5. When separating three *Meloidogyne* species and three *M. incognita* races, the minimum distance lied between *M. arenaria* and *M. incognita* race 1 ($D^2 = 15.7$, $P < 0.0001$). By incorporating these profiles into a Sherlock® Analysis Software library, the FAME method can be used to distinguish among four *Meloidogyne* species to provide an alternative source of identification.

*Key words:* biochemistry, FAME analysis, identification, *Meloidogyne* spp., *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne* races, root-knot nematodes.

## RESUMEN

Sekora, N. S., K. S. Lawrence, P. Agudelo, E. van Santen, and J. A. McInroy. 2010. Diferenciación de especies de *Meloidogyne* por análisis de metil ésteres de ácidos grasos. Nematropica 40:163-175.

El análisis de metil ésteres de ácidos grasos (FAME) se puede usar como método de diferenciación de géneros de nematodos fitoparásitos. El objetivo de este estudio fue evaluar este sistema para identificar especies. Se extrajeron y analizaron ácidos grasos de muestras con 1000 individuos cada una de *Meloidogyne arenaria* (raza 2), *M. hapla*, *M. incognita* (razas 1, 2, y 3), y *M. javanica*. Los perfiles generados por Sherlock® Analysis Software se analizaron estadísticamente y se encontró que los perfiles para cada especie y raza de *Meloidogyne* son significativamente diferentes. Las cuatro especies de *Meloidogyne* se separaron con una distancia de Mahalanobis al cuadrado ($D^2$) mínima de 15.9 entre *M. incognita* y *M. arenaria* ($P < 0.0001$). Los valores de $D^2$ entre razas de *M. incognita* fueron significativos ($P < 0.0001$) con una distacia minima entre *M. incognita* raza 1 y *M. incognita* raza 2 de 17.5. Al separar las especies de *Meloidogyne* y las tres razas de *M. incognita*, la menor distancia se observó entre *M. arenaria* y *M. incognita* raza 1 ($D^2 = 15.7$, $P < 0.0001$). Si se incorporan estos perfiles en una biblioteca de Sherlock® Analysis Software, el sistema FAME se puede usar para distinguir estas cuatro especies de *Meloidogyne*, brindando así un método alternativo de identificación.

*Palabras claves:* bioquímica, Análisis de metil ésteres de ácidos grasos, identificación, *Meloidogyne* spp., *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, nematodos agalladores.

## INTRODUCTION

There are four species within the genus *Meloidogyne* (Chit.), the root-knot nematodes that cause the majority of the known economic damage to agricultural crops across the United States (Chitwood, 1949; Bridge and Starr, 2007). Damage to cotton crops in the United States from the root-knot nematode for 2008 was estimated at $24,145,182, accounting for 84% of the total cotton losses from nematode damage and 23% of total cotton disease losses (Blasingame *et al.*, 2009). Certain species of *Meloidogyne* are host-specific, but the host potential for *Meloidogyne* spp. covers most cropping plants (Thorne, 1961; Bridge and Starr, 2007). Identification of these nematodes is complicated by races within *Meloidogyne* species. Races are host-specific; for instance, two races within *M. incognita* may both infect tomato, but only one of these two races may infect tobacco. To identify *Meloidogyne* species, the methods most often used are microscopic study of perineal patterns (Hooper, 1986), esterase phenotypes (Dickson, *et al.*, 1971; Esbenshade and Triantaphyllou, 1985; Venkatachari *et al.*, 1991), and PCR methods (Powers and Harris, 1993); the North Carolina Differential Host Test (Taylor and Sasser, 1978) is used for race identification. These methods of identification are time-consuming, require trained individuals to perform the identification, and may require life stages not found in soil samples. Other methods using mitochondrial DNA have been proposed (Okimoto *et al.*, 1991), but these methods have not been adopted by diagnostic laboratories for identification of *Meloidogyne* samples. In our lab, species and races within the *Meloidogyne* genus were identified based on species identifications of Chitwood (1949) and race identifications of Taylor and Sasser (1978).

Studies comparing species within *Meloidogyne* and *Caenorhabditis* indicate that the fatty acid profiles of these species are distinct and vary enough for differentiation (Hutzell and Krusberg, 1982; Krusberg *et al.*, 1973). It should be possible to further differentiate additional species within *Meloidogyne*, and possibly races within species, based on fatty acid profiles.

The objectives for this study are to 1) determine if *M. arenaria* (Chit.), *M. hapla* (Chit.), *M. incognita* (Chit.), and *M. javanica* (Chit.) can be differentiated using FAME analysis, 2) establish if races within *Meloidogyne* species can be identified with FAME analysis, 3) evaluate the combined statistical separation of four *Meloidogyne* species and three *M. incognita* races, and 4) assess identification of species and races within the *Meloidogyne* genus using the Sherlock® Analysis Software.

## MATERIALS AND METHODS

A stock population of *M. incognita* race 3 was collected from the Plant Breeding Unit of the E. V. Smith Research Center in Shorter, AL and increased at the Auburn University Plant Science Research Center greenhouses on *Lycopersicon esculentum* Mill. var. 'Roma' in 500 $cm^3$ polystyrene pots. The remaining populations in this study (*M. incognita* [Chit.] races 1 and 2 [Hartmann and Sasser], *M. arenaria* (race 2) [Hartmann and Sasser], *M. hapla*, and *M. javanica*) were increased on *L. esculentum* var. 'Rutgers' at the Clemson University greenhouses.

*Nematode extraction:* Second stage juvenile life stages (J2s) of *Meloidogyne* populations were extracted from the soil of the stock pots using gravity screening, and eggs of all species and races were extracted from root tissue using NaOCl. Both extractions for each species were then combined and centrifuged utilizing a sucrose gradient to remove any remaining debris. Extractions for all species were enumerated to determine the number of eggs and J2s in samples.

*Sample preparation and fatty acid extraction:* Samples analyzed contained a total of 1000 individuals from one of the six *Meloidogyne* species or races. Each *Meloidogyne* population was replicated 20 times for a total of 120 samples. Fatty acids were extracted from samples using the method described by Sasser (1990). After the extraction procedure was completed, the organic solvent was transferred to sample vials and allowed to evaporate under a fume hood. Dried samples were reconstituted in 75 µL of organic extraction solvent and transferred to spring-vial inserts for each sample vial. Vials were sealed and stored at -20°C until analysis.

Samples were analyzed for fatty acid composition by a HP 5890 automated gas chromatography system (Agilent Technologies, city and state in the U.S. or locations in other countries) equipped with an Ultra 2 Cross-linked 5% Phenyl Methyl Siloxane column; 2.0 µL of sample was injected into the column for each analysis. Sample data from the Sherlock® Sequencer Software included total response of each sample (mV) and the response for each detected fatty acid. Fatty acid percentages were calculated from the proportion of each fatty acid within the sample; these percentages were used to create a fatty acid profile for each nematode sample.

*Statistical Analysis:* For this experiment, we made three separate comparisons (1) comparing the four *Meloidogyne* species, 2) comparing the three *M. incognita* races, and 3) comparing all six species and races. For the first comparison, class values were defined by "species," in which all analyses were grouped by their respective *Meloidogyne* species; *M. incognita* races were pooled in this analysis to represent variation within the species. To compare the races of *M. incognita*, the three races were compared to one another using the "race" class. A complete analysis of all species and races within species was conducted by using "species and race" as a class for all species and races; if only a single race within a species was analyzed, its "race" classification was the same as its "species" classification.

The STEPDISC (SAS version 9.1.3; SAS Institute, Inc.) procedure was used to analyze the expression of each fatty acid across all samples to determine which fatty acids contributed significantly to the differentiation among classes; classes for each experiment were dependent upon the character being analyzed. In this case, classes were either "species" or "race" depending on the comparison. The STEPDISC procedure determined fatty acids significant for discrimination among classes based on the ANOVA test F value of a selected fatty acid (Johnson, 1998). The compiled list of fatty acids was used for class differentiation with the CANDISC procedure. The CANDISC procedure provided canonical discriminant analysis (CDA) of the fatty acid profiles for each nematode sample within its respective categorical class.

*Sherlock® Analysis Software:* A library was developed using the Sherlock® Analysis Software by creating entries from fatty acid profiles of the *Meloidogyne* species and races developed in this study. To determine the usefulness and validity of the newly created library entries with this software, individual samples were compared against their respective composite profiles to create comparison and similarity matrices for each *Meloidogyne* species and race. Identification reports were also used to evaluate identification accuracy using the "First choice" and "First Second choice" methods.

## RESULTS

*Meloidogyne Species:* The four *Meloidogyne* species had many of the same fatty acids present (Table 1), but the percentages of each fatty acid varied significantly among

Table 1. Fatty acid profiles for four *Meloidogyne* species, including three *M. incognita* races. Means are listed in order by fatty acid chain length, location of the double bond, and functional group.

| Fatty Acid | *M. arenaria* | *M. hapla* | *M. incognita* race 1 | *M. incognita* race 2 | *M. incognita* race 3 | *M. javanica* |
|---|---|---|---|---|---|---|
| 10:0 | 0.70 | 0.16 | 0.05 | 0.08 | — | 0.38 |
| 12:0 2OH | 3.28 | 1.37 | 2.14 | 3.55 | 8.01 | 2.05 |
| 14:0 | 0.52 | 0.90 | 0.33 | — | 0.14 | 0.48 |
| 14:0 2OH | 0.19 | 0.03 | — | — | 0.06 | 0.18 |
| 15:0 ISO | 0.98 | 1.27 | 0.60 | 0.38 | 0.48 | 0.91 |
| 16:0 | 7.39 | 7.19 | 7.37 | 8.74 | 18.66 | 8.69 |
| 16:1 ω5c | 0.68 | 0.55 | 0.44 | 0.10 | — | 4.58 |
| 16:1 ω7c | 1.85 | 1.36 | 1.18 | 2.84 | 0.11 | 1.32 |
| 17:0 ANTEISO | — | — | — | — | 1.08 | — |
| 17:0 ISO | 0.79 | 0.82 | 0.24 | — | 0.08 | 0.90 |
| 18:0 | 13.34 | 12.96 | 14.38 | 15.09 | 40.65 | 11.49 |
| 18:0 3OH | 0.03 | 0.25 | 0.05 | — | — | 0.26 |
| 18:1 ω5c | 1.88 | 1.54 | 1.65 | 0.91 | 0.06 | 1.72 |
| 18:1 ω7c | 57.45 | 58.89 | 58.94 | 57.08 | 20.54 | 54.50 |
| 18:1 ω9c | 1.61 | 2.07 | 2.23 | 2.18 | 0.14 | 2.07 |
| 18:2 ω6,9c/18:0 ANTE | 1.94 | 2.55 | 2.93 | 2.54 | 0.11 | 1.97 |
| 18:3 ω6c (6,9,12) | 0.35 | 0.63 | — | 0.21 | 0.26 | 0.50 |
| 19:1 ISO I | 1.63 | 1.44 | 1.35 | 0.95 | 0.95 | 1.54 |
| 20:0 | 1.56 | 1.67 | 2.02 | 1.86 | 1.81 | 1.41 |
| 20:0 ISO | 0.06 | 0.34 | 0.02 | — | — | 0.40 |
| 20:1 ω7c | 3.45 | 3.70 | 3.94 | 3.50 | 3.11 | 4.15 |
| 20:2 ω6,9c | 0.00 | 0.13 | 0.06 | — | — | 0.19 |
| 20:4 ω9,12,15c | 0.01 | 0.19 | — | — | — | 0.24 |
| unknown 18.814 | — | — | — | — | 8.31 | — |

all species ($D^2 \geq 15.9$, $P < 0.0001$). This variation was best observed between *M. incognita* and *M. javanica* (Fig.1); these two species differed the most in their expression of the fatty acids 18:1 ω7c and 18:0. In *M. javanica*, 18:1 ω7c was present at 54.5%, which was similar to *M. arenaria* (57.5%) and *M. hapla* (58.9%) but greater than *M. incognita* (45.5%). The difference in 18:0 between *M. javanica* and *M. incognita* was primarily due to the higher percentage of 18:0 found in *M. incognita* (23.4%), which was twice that of *M. javanica* (11.5%). Differences between *M. arenaria* and *M. hapla* were less pronounced than those between *M. incognita* and *M. javanica*. Most fatty acids varied by less than 0.5%. However, 12:0 2OH was found in *M. arenaria* (3.3%) at more than double the percentage found in *M. hapla* (1.4%) and 18:1 ω7c was expressed slightly higher in *M. hapla* (58.9%) than *M. arenaria* (57.5%; Table 1).

Ten fatty acids were significant ($r \geq |0.757|$) for delineation along the first canonical dimension and defined 66.3% of the overall multivariance (Table 2). Of

Table 2. Phenotypic canonical correlation of fatty acids for the three canonical discriminant functions of FAME profile analysis for four *Meloidogyne* species. Eigenvalue, cumulative percent of total variance, and canonical correlation are listed for each canonical function. Fatty acids listed were determined significant by the STEPDISC procedure. Correlation values are determined to be significant if $r \geq |0.750|$.

| | Response variable | Discriminant variate | | |
|---|---|---|---|---|
| No. | Fatty acid variable | CAN 1 | CAN 2 | CAN 3 |
| 1 | 10:0 | 0.594 | 0.360 | -0.719 |
| 2 | 12:0 2OH | -0.809 | -0.582 | -0.080 |
| 3 | 14:0 | 0.575 | 0.806 | 0.142 |
| 4 | 14:0 2OH | 0.757 | -0.002 | -0.654 |
| 5 | 15:0 ISO | 0.640 | 0.768 | 0.039 |
| 6 | 16:1 ω5c | 0.965 | -0.262 | -0.007 |
| 7 | 16:1 ω7c | 0.551 | 0.621 | -0.557 |
| 8 | 17:0 ISO | 0.844 | 0.516 | -0.146 |
| 9 | 18:0 3OH | 0.849 | 0.325 | 0.416 |
| 10 | 18:1 ω5c | 0.790 | 0.523 | -0.320 |
| 11 | 18:1 ω7c | 0.735 | 0.659 | -0.162 |
| 12 | 18:1 ω9c | 0.865 | 0.495 | 0.080 |
| 13 | 20:0 ISO | 0.893 | 0.292 | 0.341 |
| 14 | 20:2 ω6,9c | 0.906 | 0.094 | 0.413 |
| 15 | 20:4 ω6,9,12,15c | 0.886 | 0.231 | 0.401 |
| | Eigenvalue | 6.02 | 2.12 | 0.95 |
| | Cumulative % | 66.3 | 89.6 | 100.0 |
| | Canonical Correlation | 0.93 | 0.82 | 0.70 |

these fatty acids, 16:1 ω5c was the highest correlated along CAN 1 ($r = |0.965|$; Table 2) and separated *M. incognita* (0.27%) from the other species, primarily *M. javanica* (4.58%; Table 1). The three fatty acids i) 20:2 ω6,9c, ii) 20:0 ISO, and iii) 20:4 ω6,9,12,15c were all found in greater concentrations in *M. javanica* than the other three species (Table 1). *Meloidogyne javanica* and *M. hapla* had the same mean concentration of 18:1 ω9c (2.07%), which was higher than both *M. arenaria* (1.61%) and *M. incognita* (1.52%; Table 1). Both fatty acids 14:0 2OH and 18:1 ω5c were found in the greatest concentration in *M. arenaria* (0.19% and 1.88%, respectively); *M. javanica* had the highest concentration of both 17:0 ISO (0.90%) and 18:0 3OH (0.26%). *Meloidogyne incognita* had the greatest concentration of 12:0 2OH (4.57%), followed by *M. arenaria* (3.28%), *M. javanica* (2.05%), and *M. hapla* (1.37%; Table 1).

The second canonical dimension described 23.3% of the total multivariance among species. Two fatty acids were significant ($r \geq |0.768|$; Table 2) along the second canonical dimension. Both 14:0 and 15:0 ISO were found at the highest concentration in *M. hapla* (0.90% and 1.27%, respectively) and were lowest in *M. incognita* (0.23% and 0.49%, respectively; Table 1). The concentrations of these two fatty acids

were also higher in *M. arenaria* (0.52% and 0.98%, respectively) than *M. javanica* (0.48% and 0.91%, respectively; Table 1).

There was enough variation in the third canonical dimension to separate *M. hapla* from *M. arenaria* and describe the remaining 10.4% of multivariance, though there were no fatty acids determined to be significant ($r \leq |0.719|$; Table 2) for this separation (Fig. 1).

*Meloidogyne incognita Races:* Fatty acid expression varied significantly ($D^2 \geq 17.5$, $P < 0.0001$; Fig. 2) among the three races of *M. incognita* studied. As was the case with the four *Meloidogyne* species, many of the same fatty acids were observed among the three races but each fatty acid was expressed at different concentrations among those races (Table 1). The fatty acid concentrations of *M. incognita* race 1 and *M. incognita* race 2 were similar in the fatty acids detected and their respective concentrations, while *M. incognita* race 3 concentrations were more distinct from *M. incognita* race 1 and *M. incognita* race 2 in the fatty acids identified and their relative abundance. Though there were three fatty acids (14:0 2OH, 17:0 ANTEISO, and unknown peak 18.814) that were found in *M. incognita* race 3 and not *M. incognita* race 1 or *M. incognita* race 2 and five (10:0, 16:1 ω5c, 18:0 3OH, 20:0 ISO, and 20:2 ω6,9c) found in *M. incognita* race 1 or *M. incognita* race 2 and not *M. incognita* race 3, most differences among the profiles could be observed by the expression of four fatty acids (12:0 2OH, 16:0, 18:0, and 18:1 ω7c; Table 1). These four fatty acids had similar expression between *M. incognita* race 1 and *M. incognita* race 2 but were very different in *M. incognita* race 3. *Meloidogyne incognita* race 3 had nearly twice the concentration of 12:0 2OH (8.01%), 16:0 (18.66%), and 18:0 (40.65%) than *M. incognita* race 1 (2.14%, 7.37%, and 14.38%) or *M. incognita* race 2 (3.55%, 8.74%, and 15.09%). In contrast, 18:1 ω7c concentrations in *M. incognita* race 1 (58.94%) and *M. incognita* race 2 (57.08%) were three times the concentration of *M. incognita* race 3 (20.54%; Table 1).

Using CDA, eight fatty acids were significant for differentiating *M. incognita* race 3 from *M. incognita* race 1 and *M. incognita* race 2 along the first canonical dimension (87.5% of total multi variance; Table 3). All eight of these fatty acids were highly correlated along CAN 1 ($r \geq |0.875|$), but five of these (unknown 18.814, 18:1 ω9c, 18:0, 18:1 ω7c, and 18:2 ω6,9c/18:0 ANTE) were correlated along CAN 1 at greater than |0.975| (Table 3). Of these fatty acids, the unknown 18.814 peak was only detected in *M. incognita* race 3 samples (Table 1). The three fatty acids 18:1 ω9c, 18:1 ω7c, and 18:2 ω6,9c/18:0 ANTE were found at higher concentrations in *M. incognita* race 1 and *M. incognita* 2 than *M. incognita* race 3, whereas 18:0 in *M. incognita* race 3 (40.65%; Table 1) was nearly three times that of both *M. incognita* race 1 (14.38%) and *M. incognita* race 2 (15.09%). When considering the other three significant fatty acids, 12:0 2OH was more abundant in *M. incognita* race 3 (8.01%) than *M. incognita* race 1 (2.14%) and *M. incognita* race 2 (3.55%), but both 16:1 ω7c and 18:1 ω5c were higher in *M. incognita* race 1 (1.18% and 1.65%, respectively) and *M. incognita* race 2 (2.84% and 0.91%) than *M. incognita* race 3 (0.11% and 0.06%; Table 1).

The remaining 12.5% of multi variance was described by CAN 2, which helped to further differentiate *M. incognita* races 1, 2, and 3. Four fatty acids (14:0, 15:0 ISO, 17:0 ISO, and 19:1 ISO I) were significant in this separation ($r \geq |0.870|$), but three of these, 14:0, and 15:0 ISO, and 19:1 ISO I were nearly perfectly correlated along CAN 2 ($r \geq |0.991|$; Table 3). Among the four significant fatty acids, 14:0 and 17:0 ISO were not

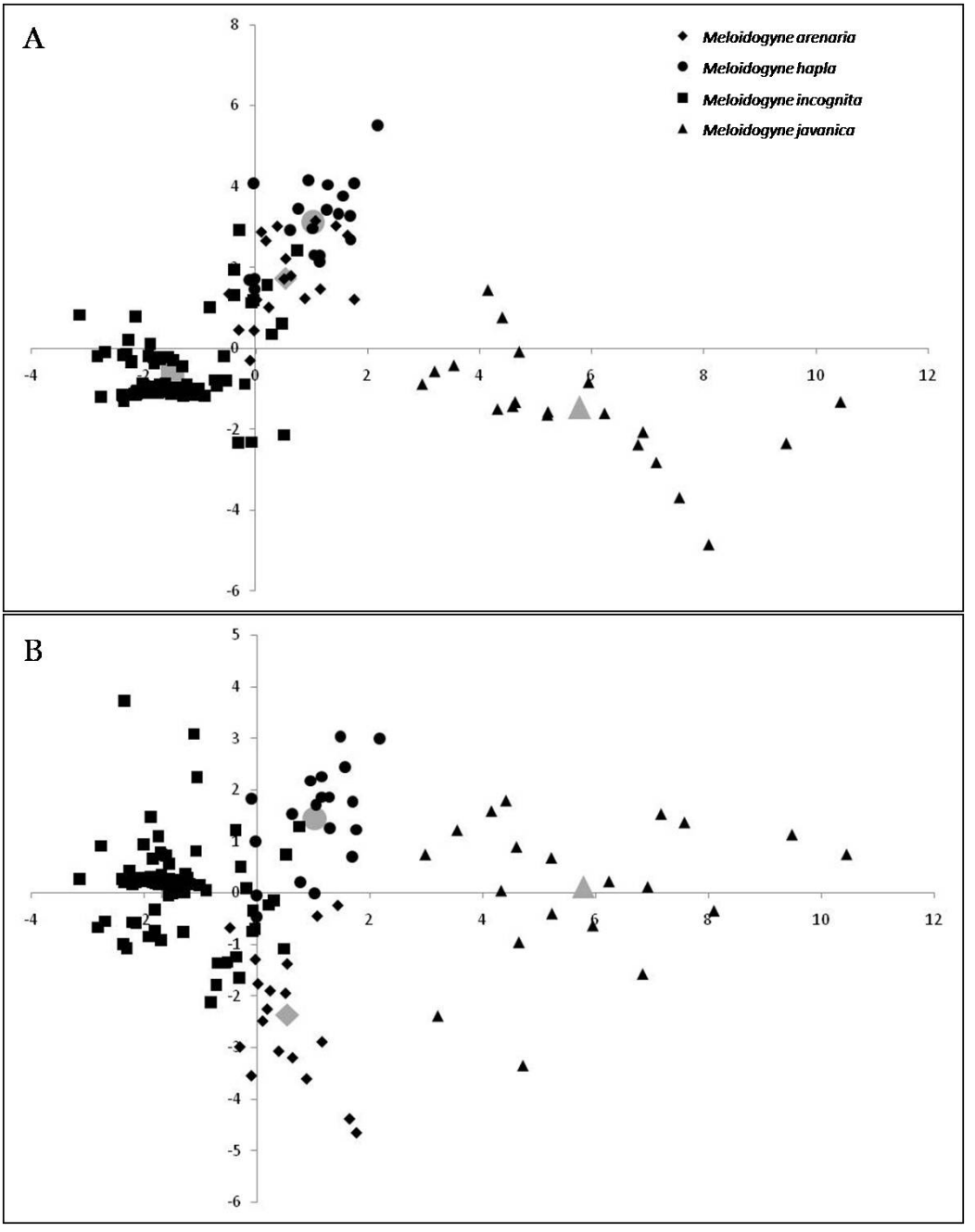


Fig. 1. Canonical distribution of four *Meloidogyne* species. Graphs represent A) x-axis versus y-axis (x, y) plot of sample canonical values with class means represented by large, grey points and B) x-axis versus z-axis (x, z) plot of sample canonical values with class means represented by large, grey points.

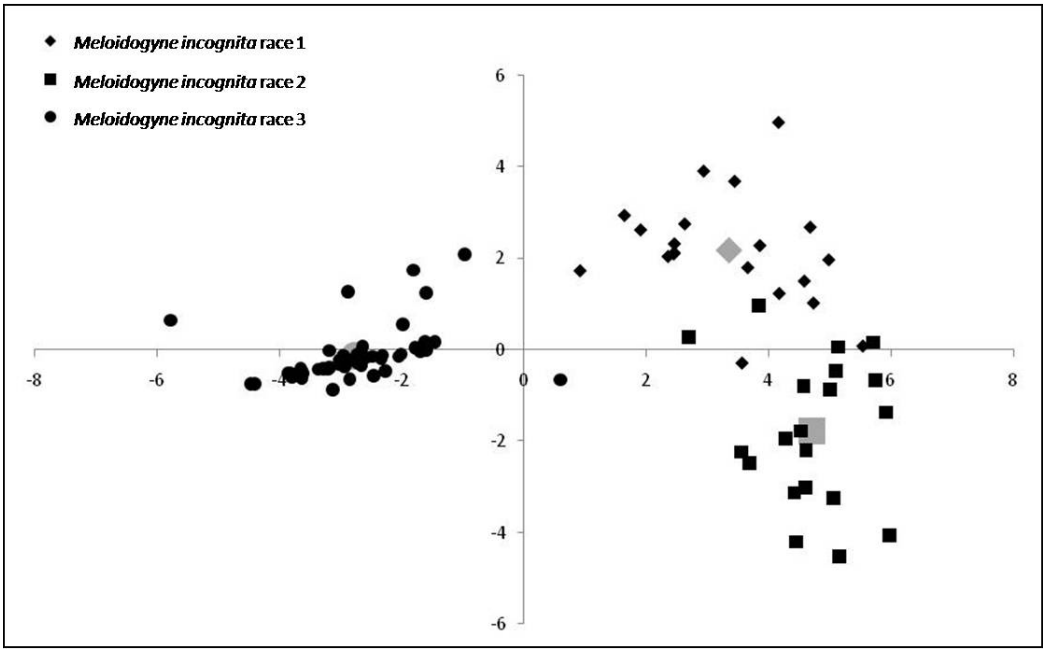


Fig. 2. Sample canonical distribution for three *Meloidogyne incognita* races. The x-axis represents the first canonical dimension and the y-axis represents the second canonical dimension. Class means are represented by large, grey points.

found in all three races; concentrations of these two fatty acids were 0.33% and 0.24%, respectively in *M. incognita* race 1 and 0.14% and 0.08% in *M. incognita* race 3, but both were absent from *M. incognita* race 2 (Table 1). The concentration of 15:0 ISO was higher in *M. incognita* race 1 (0.60%) than in *M. incognita* race 2 (0.38%) or *M. incognita* race 3 (0.48%); 19:1 ISO I was expressed lower in *M. incognita* race 3 and *M. incognita* race 2 (both at 0.95%) than *M. incognita* race 1 (1.35%; Table 1).

*Meloidogyne Species and Races:* By combining the fatty acid profiles we developed for *M. arenaria* (race 2), *M. hapla*, *M. javanica*, *M. incognita* race 1, *M. incognita* race 2, and *M. incognita* race 3, it was possible to differentiate all six populations ($D^2 \geq 15.7$, $P < 0.0001$). The fatty acid profiles of five *Meloidogyne* populations were similar in composition, but the profile of *M. incognita* race 3 appeared to be less similar to the other profiles. The percentage of 18:1 ω7c in *M. incognita* race 3 was 20.54% (Table 1); the percentage of this fatty acid in other populations ranged from 54.50% in *M. javanica* to 58.94% in *M. incognita* race 1 (Table 1). Also, concentrations of 16:0 and 18:0 in *M. incognita* race 3 (18.66% and 40.65%) were more than twice as high as any other *Meloidogyne* population studied (Table 1).

Using CDA confirmed that *M. incognita* race 3 was the most distinct profile analyzed; the average $D^2$ for *M. incognita* race 3 was 72.3, ranging from 57.6 to 101.5 between canonical means for *M. incognita* race 1 and *M. javanica*, respectively (Table 4). The first three canonical dimensions described 93.0% of the total multivariance among *Meloidogyne* populations. The first

Table 3. Phenotypic canonical correlation of fatty acids for the two canonical discriminant functions of FAME profile analysis for three *Meloidogyne incognita* races. Eigenvalue, cumulative percent of total variance, and canonical correlation are listed for each canonical function. Fatty acids listed were determined significant by the STEPDISC procedure. Correlation values are determined to be significant if $r \geq |0.750|$.

| | Response variable | Discriminant variate | |
|---|---|---|---|
| No. | Fatty acid variable | CAN 1 | CAN 2 |
| 1 | 12:0 2OH | -0.954 | -0.299 |
| 2 | 14:0 | 0.004 | 1.000 |
| 3 | 15:0 ISO | -0.081 | 0.997 |
| 4 | 16:1 ω5c | 0.686 | 0.728 |
| 5 | 16:1 ω7c/15 ISO | 0.925 | -0.379 |
| 6 | 17:0 ISO | 0.131 | 0.991 |
| 7 | 18:0 | -0.989 | -0.145 |
| 8 | 18:1 ω5c | 0.875 | 0.484 |
| 9 | 18:1 ω7c | 0.987 | 0.159 |
| 10 | 18:1 ω9c | 0.990 | 0.144 |
| 11 | 18:2 ω6,9c/18:0 ANTE | 0.975 | 0.222 |
| 12 | 19:1 ISO I | 0.493 | 0.870 |
| 13 | unknown 18.814 | -0.992 | -0.128 |
| | Eigenvalue | 11.74 | 1.68 |
| | Cumulative % | 87.5 | 100.0 |
| | Canonical Correlation | 0.96 | 0.80 |

canonical dimension explained 58.3% of the total multivariance and primarily separated *M. incognita* race 3 from *M. javanica* by 12:0 2OH with $r \geq |0.970|$. Six fatty acids (18:1 ω7c, 12:0 2OH, 18:1 ω9c, 18:1 ω5c, 18:2 ω6,9c/18:0 ANTE, and 16:1 ω7c) were responsible for the separation of populations along the first canonical dimension (Table 4). All of these fatty acids except 12:0 2OH were found in lower concentrations in the five *Meloidogyne* populations (from 1.37% in *M. hapla* to 3.55% in *M. incognita* race 2) compared to *M. incognita* race 3, within which 12:0 2OH (8.01%) was expressed at more than twice the concentration of any other population (Table 1).

Even though no significant fatty acids were observed in CAN 2 ($r \leq 0.709$) or CAN 3 ($r \leq |0.687|$; Table 4), separation along these dimensions was observed. CAN 2 was responsible for separating *M. incognita* race 2 and *M. javanica* while CAN 3 primarily distinguished *M. hapla* and *M. javanica* (Fig.3).

*Identification using the Sherlock® Analysis Software:* By analyzing samples of each *Meloidogyne* species with the library entries developed from this study, it was possible to correctly identify 90.6% of the samples. Identification accuracy was greater than 90% for *M. hapla* (94.4%), *M. incognita* (90.3%), and *M. javanica* (100%), but was reduced in *M. arenaria* (77.8%). The reduction in *M. arenaria* was caused by misidentification to *M. hapla* in 16.7% of samples. *Meloidogyne incognita* also mismatched

Table 4. Phenotypic canonical correlation of fatty acids for the two canonical discriminant functions of FAME profile analysis for four *Meloidogyne* species, including three *Meloidogyne incognita* races. Eigenvalue, cumulative percent of total variance, and canonical correlation are listed for each canonical function. Fatty acids listed were determined significant by the STEPDISC procedure. Correlation values are determined to be significant if $r \geq |0.750|$.

| | Response variable | Discriminant variate | | |
|---|---|---|---|---|
| No. | Fatty acid variable | CAN 1 | CAN 2 | CAN 3 |
| 1 | 10:0 | 0.601 | 0.212 | 0.036 |
| 2 | 12:0 2OH | -0.970 | -0.012 | -0.208 |
| 3 | 14:0 | 0.550 | 0.436 | 0.687 |
| 4 | 14:0 2OH | 0.247 | 0.605 | -0.226 |
| 5 | 15:0 ISO | 0.581 | 0.457 | 0.595 |
| 6 | 16:1 ω5c | 0.572 | 0.709 | -0.412 |
| 7 | 16:1 ω7c | 0.799 | -0.519 | -0.168 |
| 8 | 17:0 ISO | 0.684 | 0.573 | 0.298 |
| 9 | 18:0 3OH | 0.628 | 0.641 | 0.206 |
| 10 | 18:1 ω5c | 0.943 | 0.100 | 0.210 |
| 11 | 18:1 ω7c | 0.972 | -0.183 | 0.145 |
| 12 | 18:1 ω9c | 0.969 | -0.166 | 0.046 |
| 13 | 18:2 ω6,9c/18:0 ANTE | 0.924 | -0.271 | 0.165 |
| 14 | 20:0 ISO | 0.610 | 0.677 | 0.127 |
| 15 | 20:2 ω6,9c | 0.638 | 0.683 | 0.028 |
| 16 | 20:4 ω6,9,12,15c | 0.564 | 0.690 | 0.073 |
| | Eigenvalue | 14.08 | 5.91 | 2.48 |
| | Cumulative % | 58.3 | 82.7 | 93.0 |
| | Canonical Correlation | 0.97 | 0.92 | 0.84 |

8.3% of samples to *M. hapla* and 1.4% to *M. arenaria*. The remaining misidentification of samples in *M. arenaria* and *M. hapla* occurred because 5.6% of the samples in each species could not be identified.

The samples of the three races of *M. incognita* were correctly identified with 80.5% accuracy. Samples of *M. incognita* race 3 were identified with 100% accuracy. For race 1 samples, correct identification occurred at 64.7% accuracy; 5.9% of race 1 samples were identified as *M. incognita* race 2, while 29.4% were identified as either *M. arenaria* or *M. hapla*. Similarly, *M. incognita* race 2 samples were mismatched to *M. incognita* race 1 (7.7%), *M. incognita* race 3 (7.7%), and *M. arenaria* or *M. hapla* (7.7%), but 76.9% of the samples were correctly identified to the *M. incognita* race 2 library entry. Though there was some misidentification of samples, the correct *M. incognita* race was still identified greater than 64% of the time.

## DISCUSSION

We were able to clearly identify all four *Meloidogyne* species from each other using FAME analysis. The fatty acid profile generated for *M. javanica* was similar to the

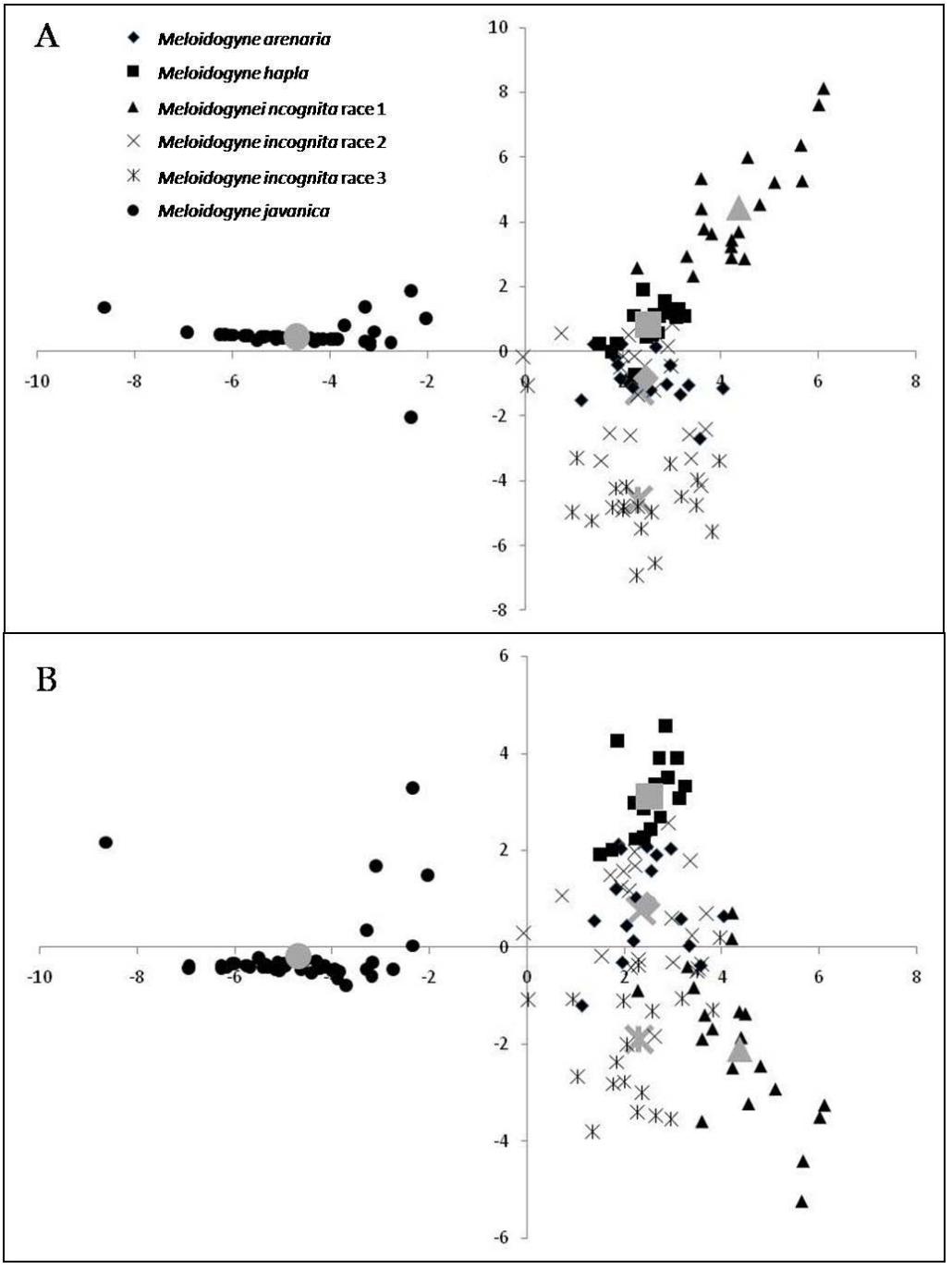


Fig. 3. Canonical distribution of four *Meloidogyne* species, including three races of *M. incognita*. Graphs represent A) x-axis versus y-axis (x, y) plot of sample canonical values with class means represented by large, grey points and B) x-axis versus z-axis (x, z) plot of sample canonical values with class means represented by large, grey points.

profile reported by Chitwood and Krusberg (1981), but there were variations in the percentages of fatty acids observed. The same pattern was also observed comparing our fatty acid profiles for *M. incognita* and *M. arenaria*; percentages of fatty acids present varied from those reported by Krusberg *et al.* (1973), but the same fatty acids were found in our study. The differences in percentages may be due to the advancement of technology since the previous studies were performed or the methods on which the nematode isolates were increased. As these and other studies indicated (Hutzell and Krusberg, 1982), fatty acid profiles among species within *Meloidogyne* expressed the same fatty acids, but the expression of those fatty acids was not uniform among species. Enough differences were observed among species in our study to separate the four *Meloidogyne* species and three *M. incognita* races studied using the Sherlock® Analysis Software with 85.6% overall accuracy. The species within *Meloidogyne* share an average of 17% similarity among fatty acid profiles; this similarity increases to only 18% within races of *M. incognita*. Because of these low similarities and the high degree of identification accuracy, identifying species and races of *Meloidogyne* with FAME analysis may be a practical means of identification to reinforce other methods of identification.

The Sherlock® Microbial Identification System has been used to identify bacterial samples since 1985. FAME analysis with this software has revolutionized bacterial identification in a way that has increased the efficiency of diagnostic laboratories around the world. Since many plant disease diagnostic laboratories already have a FAME analysis system, it should be easy to incorporate nematode identification with this system. By using the developed library of nematode fatty acid profiles, identification of *Meloidogyne* species and races would be much faster and more economically feasible than traditional methods that can require more time and resources.

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