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Using FAME Analysis to Compare, Differentiate, and Identify Multiple Nematode Species

Nicholas S. Sekora,[1] Kathy S. Lawrence,[1] Paula Agudelo,[2] Edzard van Santen,[3] John A. McInroy[1]

Abstract: We have adapted the Sherlock® Microbial Identification system for identification of plant parasitic nematodes based on their fatty acid profiles. Fatty acid profiles of 12 separate plant parasitic nematode species have been determined using this system. Additionally, separate profiles have been developed for *Rotylenchulus reniformis* and *Meloidogyne incognita* based on their host plant, four species and three races within the *Meloidogyne* genus, and three life stages of *Heterodera glycines*. Statistically, 85% of these profiles can be delimited from one another; the specific comparisons between the cyst and vermiform stages of *H. glycines, M. hapla* and *M. arenaria*, and *M. arenaria* and *M. javanica* cannot be segregated using canonical analysis. By incorporating each of these fatty acid profiles into the Sherlock® Analysis Software, 20 library entries were created. While there was some similarity among profiles, all entries correctly identified the proper organism to genus, species, race, life stage, and host at greater than 86% accuracy. The remaining 14% were correctly identified to genus, although species and race may not be correct due to the underlying variables of host or life stage. These results are promising and indicate that this library could be used for diagnostics labs to increase response time.

Key words: biochemistry, FAME analysis, identification, *Meloidogyne* spp., *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Rotylenchulus reniformis*, *Heterodera glycines*.

Fatty acid analysis has been performed on numerous types of organisms, most notably bacteria. In 1988, Myron Sasser developed a method that labeled whole-cell fatty acids of bacterial cells that could then be analyzed by an automated gas chromatograph (GC) (Kunitzky et al., 2005). This process hydrolyzes fatty acids from phospholipids, triacylglycerols, sterols, and various other lipid structures and then adds a methyl group to the carboxyl group of fatty acids which forms a methyl ester that acts as a label for the GC, which then reads and identifies those fatty acids. The system was termed FAME analysis since it utilizes fatty acid methyl esters.

Identification of fatty acids with this system is accomplished with measurements of retention time, which is the time for a specific fatty acid to pass through the GC column (Sherlock® Analysis Software, MIDI Systems, Inc.). The analysis software of the GC contains a library of retention times that it matches to the retention time of a fatty acid from an unknown sample. The percentage of each fatty acid is calculated by the proportion of response (measured in the electrical response mV) produced when it passes though the detector at the end of the column over the total response of the sample. The basic structure of a fatty acid is a carbon skeleton usually containing at least 12 carbon atoms with a carboxyl group (-COOH) at one end and a methyl group ($-CH_3$) at the other. To identify the various fatty acids, a numbering scheme is used that starts at the carboxyl carbon – the number one or α (alpha) carbon – and ending at the terminal, or ω (omega), carbon; the carboxyl carbon is labeled C-1. The number of carbon atoms (*x*) are given, followed by a colon (:) and the number of double bonds in that molecule (*y*). The double bond and any other modifications to the carbon backbone - methyl groups ($-CH_3$), hydroxyl groups (-OH) – are indicated by the number of carbon atoms from the carboxyl end (*z*). For example, a fatty acid named 18:1 ω5c (*x*:*y z*) would contain 18 carbon atoms and a single double bond, the double bond located between the 13C-14C bond. Since the double bond begins at 13C in the 18:1 ω5c example, there are five carbon atoms from 13C to the ω carbon. Therefore, the ω5c notation indicates that the double bond is located five carbon atoms from the ω carbon.

In addition to the original purpose of this system to study bacterial populations, this system has been adapted for studying fungi (Graham et al., 1995; Stahl and Klug, 1996) and a few studies have analyzed nematode fatty acid profiles (Ruess et al., 2002). However, no studies have focused on the use of the FAME system for direct differentiation and identification of plant parasitic nematodes.

By using the FAME extraction method, we have been able to develop fatty acid profiles for several species, races, and life stages of plant parasitic nematodes as well as quantify the impact of various host species on the fatty acid profiles of *Meloidogyne incognita* and *Rotylenchulus reniformis* (Sekora et al., 2008a, 2008b, 2009a, 2009b, and 2009c). The overall goal of our continuing research is to develop a library of plant parasitic nematode fatty acid profiles for use with the Sherlock® Analysis Software (MIDI, Inc) that can identify nematode samples in plant disease diagnostic laboratories at a more economical cost than current practices. To evaluate the applicability of the developed library for nematode sample identification, the twenty library entries were analyzed to quantify any overlap among profiles that would hinder identification.

Three objectives were outlined to analyze fatty acid profiles within the library using both statistical analysis and the Sherlock® Analysis Software, 1) statistical analysis of fatty acid profiles based on species classification,

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[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849.
[2]Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC 29634.
[3]Department of Agronomy and Soils, Auburn University, Auburn, AL 36849
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E-mail: lawrekk@auburn.edu
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2) additionally analyzing species fatty acid profiles while keeping any unique profiles (race, host plant, life stage) as separate profiles, and 3) using the Sherlock® Analysis Software to analyze the similarity among all 20 developed profiles.

Materials and Methods

General Culturing: Populations of *R. reniformis* (Linford and Oliveira) and *Heterodera glycines* (Ichinohe) race 3 (Golden) were collected from populations found in field sites across Alabama. *Rotylenchulus reniformis* populations were increased on *Gossypium hirsutum* (L.) cv. 'Stoneville 5599 BGRR,' *Lycopersicon esculentum* (Mill.) cv. 'Roma,' and *Glycine max* (L.) cv. 'Hutcheson.' The mixed population of *H. glycines* was increased on Croplan Genetics *G. max* cv. 'RC 4955.' A stock population of *M. incognita* race 3 (Chit.) was collected from the Plant Breeding Unit of the E. V. Smith Research Center in Shorter, AL and increased on *L. esculentum* cv. 'Roma,' *G. hirsutum* cv. 'Delta and Pine Land (DPL) 555 BGRR', and *G. max* cv. 'Hutcheson'. These populations of *R. reniformis*, *M. incognita* race 3, and *H. glycines* were increased in 500cm^3 polystyrene pots at the Auburn University Plant Science Research Center greenhouses. Pots were physically separated by Plexiglas dividers (61 cm high by 91 cm deep) to prevent the formation of mixed populations.

The remaining *Meloidogyne* species in this study (*M. arenaria* [Chit.] race 2 [Hartmann and Sasser], *M. hapla* [Chit.], *M. incognita* [Chit.] races 1 and 2 [Hartmann and Sasser], and *M. javanica* [Chit.]) were derived from populations collected around the country and reared on *L. esculentum* cv. 'Rutgers' at the Clemson University greenhouses; these populations were maintained in 45 cm clay pots and also physically separated by Plexiglas dividers. Populations of *Aphelencoides fragariae*, *Aphelenchus avenae*, *Bursaphelenchus xylophilus*, *Ditylenchus dipsaci*, *Pratylenchus penetrans*, and *Radopholus similis* were obtained from lab cultures maintained at Clemson University.

Nematode Extraction: Second stage juveniles (J2s) of *Meloidogyne* populations, juveniles and vermiform adults of *R. reniformis*, as well as J2s, mature females, and cysts of *H. glycines* were extracted from the soil of stock pots using combined gravity screening and sucrose centrifugation (Jenkins, 1964). Gravid females and eggs of all *Meloidogyne* species and races were extracted from root tissue by agitation in 0.6% NaOCl for four minutes (Hussey and Barker, 1973). Both extractions for each species and race were then combined and centrifuged utilizing a sucrose gradient to remove any remaining plant and soil debris. Individuals of *A. fragariae*, *A. avenae*, *B. xylophilus*, *D. dipsaci*, *P. penetrans*, and *R. similis* were collected by rinsing multiple plates of each species over nested sieves to remove culture and plant debris. Extractions for all species and races were enumerated using an inverted microscope to determine the number of each life stage in samples.

Fatty Acid Extraction: A total of 867 samples were prepared from races, hosts, and life stages of the 12 nematode species. Fatty acids from samples were extracted 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. The 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 an HP 5890 automated gas chromatography system (Agilent Technologies) equipped with an Ultra 2 Crosslinked 5% Phenyl Methyl Siloxane column and a flame-ionization detector; 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: Two comparisons among nematode fatty acid profiles were made in this study: 1) comparing fatty acid profiles at the species level, and 2) comparing profiles generated for each species, race, life stage, and host. Species comparisons were made by including all races, life stages, and hosts under a single "species" class for each nematode species. Total profile comparisons of various life stages, hosts, or races were classified to a "variable" class and did not combine any profiles from a species.

The STEPDISC (SAS version 9.1.3; SAS Institute, Inc) procedure was used to analyze the percentage of each fatty acid across all samples within a given class to determine which fatty acids contributed significantly to the differentiation among classes (species or variable) 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: Sample identification is dependent on the "choice" method. When a sample is analyzed with the Sherlock® Analysis Software, the sample fatty acid profile is compared to fatty acid profiles within the software's library based on fatty acids present and the percentage of each fatty acid. Library matches are ranked by similarity; the library entry that is the closest match to the sample profile is ranked the highest, followed by the next analogous entry. "First choice" identification accuracy is based on the proportion of samples that are matched correctly to the highest ranked library entry, whereas "First Second choice" accuracy is dependent on the correct library

entry being the first or second most similar entry matched. These matching methods are linked to the comparison matrices for the library; "first-choice" identifications are more accurate when the comparison percentages are closer to 100%. Identification reports for a library entry list the accuracy of matching samples in that entry to the correct fatty acid profile using the "First choice" method; any samples that missed the correct identification are further analyzed using the "First Second choice" method.

A library of nematode fatty acid profiles was developed using the Sherlock® Analysis Software by creating entries from profiles of the 20 nematode species, races, life stages, and hosts in this study. To determine the validity of the newly created library entries, individual samples were compared against their respective library entries to create comparison and similarity matrices for each library entry. Identification reports were also used to evaluate identification accuracy of samples used in creating library entries by using the "First choice" and "First Second choice" methods.

Results

Differentiation by Species: A total of 54 fatty acids were observed among the 12 nematode species studied (Table 1). Of these 54 fatty acids, an average of 11 (19%) were expressed within each nematode species profile. The maximum number of fatty acids observed in a species profile was 49 (91%) fatty acids within *R. reniformis*; a minimum of 7 (13%) fatty acids were observed within the profile of *B. xylophilus*. Most of these fatty acids (80%) were expressed at percentages less than 1.0% among all species profiles; *H. glycines* expressed 19 (35%) fatty acids above 1.0%, the most of any other profile. Two fatty acids, 16:0 and 18:1 ω7c, were observed in the highest concentrations among the nematode species. Six species, *A. fragariae*, *A. avenae*, *B. xylophilus*, *D. dipsaci*, *P. penetrans*, and *R. reniformis,* expressed 16:0 as their primary fatty acid; percentages ranged from 16.21% in *R. reniformis* to 50.26% in *B. xylophilus*. For the remaining six species, *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, *H. glycines*, and *R. similis*, 18:1ω7c was the fatty acid with the highest percentage and ranged from 38.21% in *M. incognita* to 58.89% in *M. hapla*. The fatty acid most commonly expressed at the second highest percentage was 18:0 in seven species, *A. fragariae*, *A. avenae*, *B. xylophilus*, *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica*. The expression of 18:0 in these seven species ranged from 11.49% in *M. javanica* to 27.68% in *B. xylophilus*, with a mean of 18.83%.

CDA explained 92.9% of the total multivariance in the first three canonical dimensions (Table 3). Based on this analysis, 98% of the total comparisons among species were significant ($D^2 > 3.8$, $P < 0.492$) (Table 2). The comparison between *M. hapla* and *M. javanica* was not significant (D^2=2.3, P=0.949) based on species comparisons. Thirty-four of the fatty acids were significant for differentiation among fatty acid profiles at the species level. The first canonical dimension explained 68.0% of the total multivariance (Table 3) and primarily separated *H. glycines* from the remaining fatty acid profiles (Figure 1). Two fatty acids, 20:4 ω6,9,12,15c and 16:1 ω7c, were primarily responsible for differentiation along the first canonical axis (Table 3). Both of these fatty acids were found in the highest concentrations in the *H. glycines* profile, 9.77% and 3.96%, respectively (Table 1). The four species within the *Meloidogyne* genus, *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica,* expressed both of these fatty acids, but at concentrations less than those of *H. glycines* (mean of 1.36% and 0.50%, respectively). *Pratylenchus penetrans* was the only other nematode species to express 20:4 ω6,9,12,15c, but its mean concentration (0.08%) was also lower than that of *H. glycines*. The fatty acid 16:1 ω7c was not observed in any other nematode species outside the *Meloidogyne* genus or *H. glycines*.

Five fatty acids, 12:0 2OH, 10:0, TBSA 10Methyl 18:0 peak, 16:1 cis 9, and 20:1 trans 11, explained 15.8% of the total multivariance along the second canonical dimension (Table 3). These fatty acids helped separate *R. reniformis* from the *Meloidogyne* species group and the remaining nematode species group containing *A. fragariae*, *A. avenae*, *B. xylophilus*, *D. dipsaci*, *P. penetrans*, and *R. similis* (Fig. 1). All five of these fatty acids are found in *R. reniformis* (Table 1). Three of the five fatty acids, 12:0 2OH, 10:0, TBSA 10Methyl 18:0 peak, were found in the highest concentrations in *R. reniformis,* 15.67%, 1.76%, and 0.46%, respectively. Both 16:1 cis 9 and 20:1 trans 11 were only found in *R. reniformis,* 0.14% and 2.21%, respectively.

The third canonical dimension explained 9.0% of the total multivariance (Table 3) and separated the group containing *A. fragariae*, *A. avenae*, *B. xylophilus*, *D. dipsaci*, *P. penetrans*, and *R. similis* from the *Meloidogyne* species group (Fig. 1). A single fatty acid, 18:1 ω7c, separated these groups. Within the *Meloidogyne* species, 18:1 ω7c had a mean expression of 52.26% and ranged from 38.21% in *M. incognita* to 58.89% in *M. hapla* (Table 1). This fatty acid was only found in three species, *D. dipsaci*, *P. penetrans*, and *R. similis,* from the other group. Expression of 18:1 ω7c within these profiles was 46.24% in *R. similis*, 30.55% in *D. dipsaci*, and 19.09% in *P. penetrans*.

Differentiation by Species, Race, Life Stage, and Host: Of the same 54 fatty acids observed in the species analysis, a mean of 22 (40%) fatty acids were observed within the profiles when separated based on life stage, host plant, and race (Tables 1 and 4). The fatty acid profiles of *R. reniformis* from cotton and soybean plants expressed the largest number of fatty acids, 41 (76%), among all profiles; the fatty acid profile of *B. xylophilus* contained the fewest fatty acids, only 7 (13%). An average of 17 (31%) fatty acids were expressed at percentages greater than 1.0% among all fatty acid profiles; *H. glycines*

TABLE 1. Fatty acid profiles for twelve nematode species. Each species is based on the average expression of fatty acids among all races, life stages, or hosts analyzed. Means are listed in order by fatty acid chain length, location of the double bond, and functional group.

Fatty Acid	AD	AP	BR	DT	MA	MH	MI	MJ	PT	RD	RR	SCN
10:0	–†	–	–	–	0.70	0.16	0.20	0.38	0.77	–	1.76	–
10:0 2OH	1.71	–	–	–	0.03	–	1.27	–	0.11	–	0.06	–
12:0	–	0.29	–	–	–	–	–	–	1.48	–	0.11	0.01
12:0 2OH	–	–	–	–	3.28	1.37	6.51	2.05	0.91	–	15.67	0.25
13:0	–	0.31	–	–	–	–	–	–	1.02	–	0.02	–
14:0	–	0.60	–	–	0.52	0.90	0.20	0.48	2.77	–	0.32	2.95
14:0 2OH	–	0.20	–	–	0.19	0.03	0.06	0.18	0.14	–	1.09	–
15:0 ANTEISO	–	–	–	–	–	–	0.24	–	–	–	0.19	0.54
15:0 ISO	–	–	–	–	0.98	1.27	0.41	0.91	–	–	0.32	1.58
15:1 ANTEISO A	–	–	–	–	0.19	–	–	0.05	–	–	0.07	–
16:0	34.94	42.15	50.26	40.18	7.39	7.19	15.84	8.69	36.85	–	16.21	7.52
16:0 2OH	–	0.20	–	–	–	–	0.15	–	0.28	29.24	0.01	–
16:0 3OH	–	–	–	–	–	–	0.20	–	–	–	–	–
16:0 ANTEISO	–	–	–	–	–	–	–	–	–	–	0.18	–
16:1 CIS 9	–	–	–	–	–	–	–	–	–	–	0.14	–
16:1 ω5c	–	–	–	–	0.68	0.55	8.99	4.58	–	–	0.41	5.01
16:1 ω7c	–	–	–	–	1.85	1.36	0.93	1.32	–	–	–	3.96
17:0	–	0.41	–	–	–	–	0.04	–	1.02	–	0.03	1.28
17:0 ANTEISO	–	–	–	–	–	–	0.60	–	–	–	1.32	–
17:0 ISO	–	–	–	–	0.79	0.82	0.29	0.90	–	–	0.15	0.44
17:1 ISO I/ANTEI B	–	–	–	–	–	–	–	–	–	–	0.37	2.37
18:0	26.24	19.94	27.68	12.82	13.34	12.96	20.18	11.49	12.19	–	14.32	3.15
18:0 3OH	0.38	0.75	–	1.64	0.03	0.25	0.20	0.26	–	3.72	0.07	1.04
18:0 ANTE/18:2 ω6,9c	0.99	6.99	2.34	–	0.09	–	0.44	–	–	10.91	0.19	1.94
18:0 ANTEISO/18:2c	–	–	–	–	–	–	–	–	–	–	0.28	–
18:1 CIS 11/t 9/t 6	–	–	–	–	–	–	–	–	–	–	12.46	–
18:1 CIS 9	–	–	–	–	–	–	–	–	–	–	0.86	–
18:1 TRANS 9/t6/c11	–	–	–	–	–	–	–	–	–	–	10.00	–
18:1 ω5c	0.73	–	–	–	1.88	1.54	0.54	1.72	–	–	–	1.91
18:1 ω7c	–	–	–	30.55	57.45	58.89	38.21	54.50	19.09	–	4.58	42.16
18:1 ω9c	20.50	13.21	11.94	3.22	1.61	2.07	2.03	2.07	0.01	–	0.09	3.70
18:2 CIS 9,12/18:0a	–	–	–	–	–	–	–	–	–	–	0.19	–
18:2 ω6,9c/18:0 ANTE	6.14	6.87	2.99	–	1.85	2.55	1.38	1.97	–	46.24	0.41	5.63
18:3 CIS 6,12,14	–	–	–	–	–	–	–	–	–	0.14	0.02	–
18:3 ω6c (6,9,12)	0.64	–	4.05	–	0.35	0.63	0.25	0.50	4.52	–	0.68	1.05
19:0	–	0.47	–	–	–	–	0.18	–	2.26	0.80	–	–
19:0 CYCLO ω8c	–	–	–	–	–	–	0.33	–	–	–	0.01	–
19:0 ANTEISO	–	–	–	–	–	–	–	–	0.18	–	2.00	–
19:1 ISO I	1.08	–	0.73	5.44	1.63	1.44	0.70	1.54	0.06	1.66	0.29	1.18
20:0	3.15	2.18	–	1.67	1.56	1.67	1.34	1.41	9.25	–	3.33	0.94
20:0 ISO	0.39	0.36	–	0.33	0.06	0.34	0.02	0.40	0.44	–	0.03	0.67
20:1 TRANS 11	–	–	–	–	–	–	–	–	–	0.13	2.21	–
20:1 ω7c	1.23	0.41	–	0.58	3.45	3.70	2.81	4.15	4.81	0.82	0.36	3.98
20:1 ω9c	0.39	–	–	0.75	–	–	–	0.01	0.33	–	–	0.02
20:2 ω6,9c	0.10	1.27	–	1.00	–	0.13	0.04	0.19	–	–	0.72	2.21
20:4 ω6,9,12,15c	–	–	–	–	0.01	0.19	1.57	0.24	0.08	5.20	–	9.77
TBSA 10Me18:0	–	–	–	–	–	–	–	–	–	–	0.46	0.03
unknown 10:928	–	–	–	–	–	–	–	–	–	–	0.39	–
unknown 15:549	–	–	–	–	–	–	–	–	–	–	1.30	–
unknown 16:582	–	–	–	–	–	–	0.42	–	–	–	0.48	–
unknown 18:814	0.24	–	–	1.82	–	–	8.31	–	–	–	5.97	–

AD: *Aphelenchoides fragariae*, *AP: Aphelenchus avenae*, BR: *Bursaphelenchus xylophilus*, DT: *Ditylenchus dipsaci*, MA: *Meloidogyne arenaria*, MH: *M. hapla*, MI: *M. incognita*, MJ: *M. javanica*, PT: *Pratylenchus penetrans*, RD: *Radopholus similis*, RR: *Rotylenchulus reniformis*, and SCN: *Heterodera glycines*.
† = Not Detected.

females expressed 16 (30%) fatty acids at percentages greater than 1.0%. Both 18:1 ω7c and 16:0 were the two fatty acids expressed at the highest concentrations among the 20 fatty acid profiles. The mean percentage of 18:1 ω7c observed was 35.18% across all fatty acid profiles. Nine profiles, *M. arenaria*, *M. hapla*, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 from cotton, *M. javanica*, *R. similis*, *H. glycines* cysts, and *H. glycines* J2s, expressed 18:1 ω7c as their primary fatty acid; percentages ranged from 46.24% in *R. similis* to 60.14% in *H. glycines* cysts. Across all profiles, 16:0 was expressed at a mean concentration of 20.37%, ranging from 2.77% in *H. glycines* cysts to 50.26% in *B. xylophilus*, and was the primary fatty acid observed within seven profiles, *A. fragariae*, *A. avenae*, *B. xylophilus*, *D. dipsaci*, *M. incognita* race 3 from soybean, *P. penetrans*, and *H. glycines*

TABLE 2. Squared Mahalanobis distances (D^2) and class distance probability of similarity (*P*) for canonical discriminant analysis of twelve nematode species.

	AD		AP		BR		DT		MA		MH	
	D^2	*P*	D^2	*P*	D^2	*P*	D^2	*P*	D^2	*P*	D^2	*P*
AD			26.4	<.0001	14.4	<.0001	36.0	<.0001	74.9	<.0001	70.0	<.0001
AP	26.4	<.0001			12.7	<.0001	33.7	<.0001	84.5	<.0001	77.1	<.0001
BR	14.4	<.0001	12.7	<.0001			19.0	<.0001	66.3	<.0001	61.4	<.0001
DT	36.0	<.0001	33.7	<.0001	19.0	<.0001			45.3	<.0001	40.1	<.0001
MA	74.9	<.0001	84.5	<.0001	66.3	<.0001	45.3	<.0001			3.9	0.492
MH	70.0	<.0001	77.1	<.0001	61.4	<.0001	40.1	<.0001	3.9	0.492		
MI	52.7	<.0001	56.9	<.0001	41.4	<.0001	25.5	<.0001	15.3	<.0001	14.0	<.0001
MJ	75.5	<.0001	82.9	<.0001	66.3	<.0001	44.4	<.0001	3.8	0.4962	2.3	0.9493
PT	46.5	<.0001	42.4	<.0001	31.8	<.0001	26.6	<.0001	52.3	<.0001	47.1	<.0001
RD	50.1	<.0001	45.1	<.0001	30.1	<.0001	15.9	<.0001	31.1	<.0001	26.9	<.0001
RR	77.1	<.0001	78.5	<.0001	67.8	<.0001	52.0	<.0001	41.3	<.0001	40.7	<.0001
SCN	459.4	<.0001	445.7	<.0001	446.2	<.0001	435.1	<.0001	394.9	<.0001	380.8	<.0001

	MI		MJ		PT		RD		RR		SCN	
	D^2	*P*	D^2	*P*	D^2	*P*	D^2	*P*	D^2	*P*	D^2	*P*
AD	52.7	<.0001	75.5	<.0001	46.5	<.0001	50.1	<.0001	77.1	<.0001	459.4	<.0001
AP	56.9	<.0001	82.9	<.0001	42.4	<.0001	45.1	<.0001	78.5	<.0001	445.7	<.0001
BR	41.4	<.0001	66.3	<.0001	31.8	<.0001	30.1	<.0001	67.8	<.0001	446.2	<.0001
DT	25.5	<.0001	44.4	<.0001	26.6	<.0001	15.9	<.0001	52.0	<.0001	435.1	<.0001
MA	15.3	<.0001	3.8	0.4962	52.3	<.0001	31.1	<.0001	41.3	<.0001	394.9	<.0001
MH	14.0	<.0001	2.3	0.9493	47.1	<.0001	26.9	<.0001	40.7	<.0001	380.8	<.0001
MI			15.5	<.0001	31.4	<.0001	12.4	<.0001	22.9	<.0001	404.4	<.0001
MJ	15.5	<.0001			50.1	<.0001	30.0	<.0001	43.1	<.0001	385.4	<.0001
PT	31.4	<.0001	50.1	<.0001			21.7	<.0001	47.6	<.0001	438.2	<.0001
RD	12.4	<.0001	30.0	<.0001	21.7	<.0001			39.4	<.0001	420.9	<.0001
RR	22.9	<.0001	43.1	<.0001	47.6	<.0001	39.4	<.0001			437.3	<.0001
SCN	404.4	<.0001	385.4	<.0001	438.2	<.0001	420.9	<.0001	437.3	<.0001		

AD: *Aphelenchoides fragariae*, *AP*: *Aphelenchus avenae*, BR: *Bursaphelenchus xylophilus*, DT: *Ditylenchus dipsaci*, MA: *Meloidogyne arenaria*, MH: *M. hapla*, MI: *M. incognita*, MJ: *M. javanica*, PT: *Pratylenchus penetrans*, RD: *Radopholus similis*, RR: *Rotylenchulus reniformis*, and SCN: *Heterodera glycines*.

females. The third most common fatty acid among all profiles was 18:0 (15.04%) and was found to be the second most abundant fatty acid in ten profiles, *A. fragariae*, *A. avenae*, *B. xylophilus*, *M. arenaria*, *M. hapla*, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 from cotton, *M. javanica*, and *R. similis*.

A total of 74.3% of the total multivariance was explained by the first three canonical dimensions using CDA (Table 5). Among the 20 profiles, 98% of the possible comparisons were significantly different ($D^2 > 8.57$, $P < 0.005$). Three comparisons were not significant when analyzing the twenty fatty acid profiles. These comparisons were between *M. arenaria* and *M. hapla* (D^2=6.6, *P*=0.1571), *M. hapla* and *M. javanica* (D^2=6.2, *P*=0.1725), and *H. glycines* cysts and *H. glycines* J2s (D^2=3.1, *P*=1.000). Forty-six fatty acids were determined to be significant for differentiating among the twenty fatty acid profiles. A single fatty acid, 20:4 ω6,9,12,15c, was responsible for separating profiles along the first canonical dimension (45.9% of multivariance) (Table 5). The three *H. glycines* profiles (cysts, females, and J2s) were separated from the remaining profiles along the first canonical dimension. Among the three *H. glycines* profiles, 20:4 ω6,9,12,15c was observed at 9.63%, 9.46%, and 10.22%, respectively. In comparison, *M. arenaria*, *M. hapla*, *M. incognita* race 3 from soybean, *M. javanica*, and *P. penetrans* expressed 20:4 ω6,9,12,15c at concentrations less than 1.6% (Tables 1 and 4).

The second canonical dimension explained 15.8% of the total multivariance among fatty acid profiles (Table 5). Four fatty acids, 16:1 ω7c, 19:0 cyclo ω8c, 16:0 3OH, and 16:1 ω5c, were responsible for separation along the second canonical axis and separated profiles within the *Meloidogyne* genus and *R. reniformis* from the six nematode species *A. fragariae*, *A. avenae*, *B. xylophilus*, *D. dipsaci*, *P. penetrans*, and *R. similis* (Fig. 2). Only profiles within the *Meloidogyne* genus (*M. arenaria*, *M. hapla*, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 from cotton, *M. incognita* race 3 from soybean, *M. incognita* race 3 from tomato, and *M. javanica*) and *H. glycines* (cysts, females, and J2s) had 16:1 ω7c in their profiles (Tables 1 and 4). Both 19:0 cyclo ω8c and 16:0 3OH were only found in profiles of *R. reniformis* and *M. incognita* race 3 that varied by host; 19:0 cyclo ω8c was found in *R. reniformis* and *M. incognita* race 3 when both were grown on soybean, and 16:0 3OH was found in *M. incognita* race 3 from soybean and *R. reniformis* from cotton. The fourth fatty acid, 16:1 ω5c, was found in *R. reniformis* from soybeans, the three life stages of *H. glycines*, *M. arenaria*, *M. hapla*, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 from soybean, and *M. javanica*. The percentage of this fatty acid varied among

TABLE 3. Phenotypic canonical correlation of fatty acids for the three canonical discriminant functions of FAME profile analysis for 12 nematode 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 $r \geq |0.75|$ (**bold**).

	Response variable	Discriminant variate		
No.	Fatty acid variable	CAN 1	CAN 2	CAN 3
1	10:0	-0.297	**-0.810**	0.429
2	10:0 2OH	-0.119	0.387	0.167
3	12:0	-0.079	0.210	0.225
4	12:0 2OH	-0.381	**-0.866**	0.212
5	14:0	0.556	0.026	0.103
6	15:0 ISO	0.503	-0.315	-0.586
7	15:1 ANTEISO A	-0.093	-0.211	-0.256
8	16:0	-0.368	0.688	0.557
9	16:0 3OH	-0.182	0.061	-0.544
10	16:1 CIS 9	-0.251	**-0.804**	0.515
11	16:1 ω5c	0.274	0.080	-0.705
12	16:1 ω7c	-0.160	0.114	-0.564
13	16:1 ω7c/15 iso 2OH	**0.805**	0.029	-0.510
14	17:0	0.132	0.299	0.270
15	17:0 ISO	0.333	-0.180	-0.637
16	17:1 ISO I/ANTEIB	0.036	0.089	-0.028
17	18:0	-0.570	0.377	-0.149
18	18:0 3OH	0.662	0.290	0.178
19	18:0 ANTE/18:2 ω6,9c	0.006	0.523	0.476
20	18:1 ω5c	0.727	0.098	-0.457
21	18:1 ω7c	0.418	0.180	**-0.868**
22	18:1 ω9c	0.062	0.736	0.415
23	18:2 ω6,9c/18:0 ANTE	0.637	0.532	0.211
24	18:3 ω6c (6,9,12)	0.028	0.283	0.326
25	19:0	-0.069	0.268	0.178
26	19:0 CYCLO ω8c	-0.171	0.088	-0.554
27	19:1 ISO I	0.144	0.308	-0.219
28	20:0	-0.233	-0.230	0.429
29	20:0 ISO	0.320	0.505	0.103
30	20:1 TRANS 11	-0.251	**-0.804**	0.515
31	20:1 ω7c	0.381	0.294	-0.705
32	20:1 ω9c	-0.063	0.450	0.222
33	20:4 ω6,9,12,15c	**0.997**	-0.036	0.041
34	TBSA 10Me18:0	-0.246	**-0.805**	0.516
	Eigenvalue	27.46	6.39	3.63
	Cumulative %	68.0	83.9	92.9
	Canonical Correlation	0.98	0.93	0.89

profiles from 0.10% in *M. incognita* race 2 to 26.43% in *M. incognita* race 3 from soybeans.

Four fatty acids, 18:1 TRANS 9/t6/c11, the unknown 15.549 peak, 12:0 2OH, and 18:0 ANTEISO/18:2 c, were responsible for defining 12.6% of the total multivariance along the third canonical dimension (Table 5). Differentiation along the third dimension separated *H. glycines* females from cysts and J2s as well as the *Meloidogyne* genus from the *R. reniformis* profiles (Fig. 2). Three of the four fatty acids, 18:1 TRANS 9/t6/c11, the unknown 15.549 peak, and 18:0 ANTEISO/18:2 c, were observed in three *R. reniformis* profiles and no other profile (Tables 1 and 4). Fourteen of the twenty profiles contained 12:0 2OH, but the highest percentages were found in *R. reniformis* (mean 15.67%). Profiles within the *Meloidogyne* genus expressed 12:0 2OH at an average of 4.91% across eight profiles; *H. glycines* cysts, *H. glycines* J2s, and *P. penetrans* expressed 12:0 2OH at less than 1.0% (0.16%, 0.34%, and 0.91%, respectively).

Identification with the Sherlock® Analysis Software: We were able to develop a library of fatty acid profiles containing 13 entries based on 440 samples. While some samples were mismatched depending on host, race, or species, 98.9% of the samples analyzed were correctly identified to the genus level with the Sherlock® Analysis Software (Table 6). *Heterodera* and *Rotylenchulus* correctly matched to their correct genus in 100% of samples. *Meloidogyne* genus samples were correctly identified at 98.4% to the genus level, 1.6% being matched to the *Rotylenchulus* genus. At the species level, four *Meloidogyne* species, *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica,* were identified at 90.7% accuracy (Table 6). Races one, two, and three of *M. incognita* matched correctly at 80.5% accuracy among all samples analyzed.

In samples of *M. incognita* race 3 and *R. reniformis* that were increased on different hosts, samples of these nematodes matched to the correct host 94.5% of the time (Table 6). *Meloidogyne incognita* race 3 samples could be correctly identified to host at 98.5% accuracy, with 1.5% of cotton samples matching to *M. incognita* race 1. Samples of *R. reniformis* from each host were correctly identified at 91.8% accuracy. A total of 2.0% and 4.0% of *R. reniformis* samples from cotton were identified as coming from soybean and tomato plants, respectively. Samples of *R. reniformis* from soybean were identified as tomato samples in 5.0% of samples. *Rotylenchulus reniformis* tomato samples were incorrectly identified in 13.6% of samples.

All life stages of *H. glycines* were correctly identified in 100% of samples. By comparing all 13 entries within the developed library, 91.2% of all samples were correctly identified to race, host, or life stage level. Species level identification of samples across these entries was 96.9% accurate.

DISCUSSION

FAME analysis was developed to identify bacterial species more quickly and easily than differential biochemical testing. The Sherlock® Analysis Software is currently able to accurately identify 1700 species of bacteria and yeast, many to the subspecies or strain level (Kunitsky et al., 2005). Hoping to exploit the usefulness of this system, researchers have begun to adapt this system for use with other groups of organisms, most notably fungi. FAME analysis has been used to characterize, classify, and identify more than 150 different species of fungi (Bentivenga and Morton, 1996; Graham et al., 1995; Stahl and Klug, 1996).

Fatty acid profiles of thirteen nematode species studied were distinct and characteristic using both CDA statistical analysis and the Sherlock® Analysis Software. In this study, 18:1 fatty acids were the predominant fatty acids in nine nematode fatty acid profiles, *M. arenaria*,

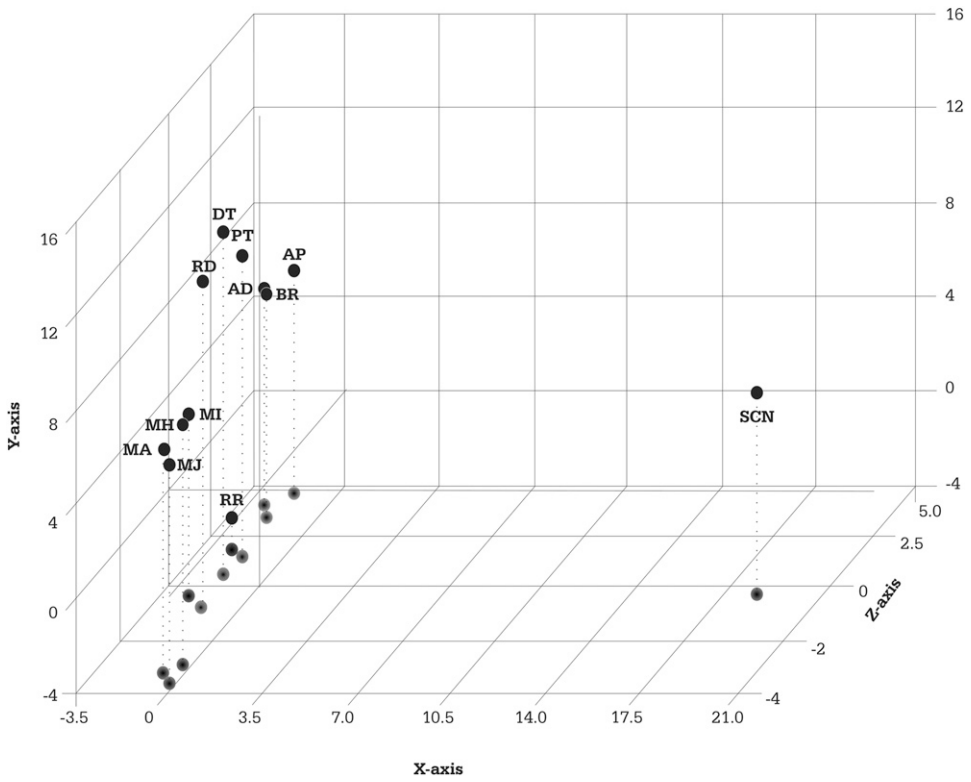

Fig. 1. Graph of canonical means for twelve nematode species. Placement is defined for the first (x-axis), second (y-axis), and third (z-axis) canonical axes by height (y-axis) and shadow (x-axis and z-axis intercept). AD: *Aphelenchoides fragariae*, AP: *Aphelenchus avenae*, BR: *Bursaphelenchus xylophilus*, DT: *Ditylenchus dipsaci*, MA: *Meloidogyne arenaria*, MH: *M. hapla*, MI: *M. incognita*, MJ: *M. javanica*, PT: *Pratylenchus penetrans*, RD: *Radopholus similis*, RR: *Rotylenchulus reniformis*, and SCN: *Heterodera glycines*.

M. hapla, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 increased on cotton, *M. javanica*, *R. similis*, *H. glycines* cysts, and *H. glycines* J2, which is similar to what Krusberg (1967) observed in his studies with other plant parasitic nematodes. Krusberg (1967) and Krusberg et al. (1973) found that the primary fatty acids in *D. dipsaci*, *P. penetrans*, *M. incognita*, and *M. arenaria* was 18:1, but in our studies we observed 16:0 as the most abundant fatty acid in *D. dipsaci*, *M. incognita* race 3 increased on soybean, and *P. penetrans*, as well as *A. avenae*, *A. fragariae*, *B. xylophilus*, and *H. glycines* females. The 16:0 fatty acid was found in the greatest abundance when samples contain less than 100 nematodes (Sekora et al., 2008). Additional studies have been conducted using FAME analysis to observe variation in fatty acid profiles of *A. avenae* and *A. composticola* caused by starvation (Chen et al., 2001). This indicates that the seven profiles generated within which 16:0 was the predominant fatty acid may be based on diluted samples, but could also be caused by variations in the feeding cycle of those nematode species. Additional samples of these species in various feeding stages would help resolve this variation and help explain differences observed among these studies.

Using stepwise comparisons and canonical analysis, we could not differentiate three comparisons, *M. arenaria* to *M. hapla, M. hapla* to *M. javanica*, and *H. glycines* cysts to *H. glycines* J2s, among the 190 profile comparisons. The profile we developed for *M. arenaria* was similar to that published by Krusberg et. al (1973), but we found the percentage of 18:0 to be twice the original reported value. The percentage of 16:0 and 18:1 fatty acids in our profile for *M. javanica* was similar to that published by Chitwood and Krusberg (1981). By comparing profiles for the four species of *Meloidogyne*, we found that fatty acids in these species were similar in percentages of fatty acids present as compared to percentages of the same fatty acids from other genera. However, fatty acid profiles of the four *Meloidogyne* species varied significantly ($P < 0.0001$) and differences could be observed when comparing within the genus (Sekora et al., 2009b). Studies by Krusberg et al. (1967) and Hutzell and Krusberg (1982) indicated that fatty acid profiles of species within the same genus could vary just as much as profiles among genera. The possibility that fatty acid profiles vary as much among species as they do among genera is promising and indicates that using fatty acid profiles as a means for identification at greater levels of specificity (race or life stage) may produce the same degree of variation.

The similarities we observed between cysts and J2s of *H. glycines* were not surprising considering that a mature cyst contains eggs with varying stages of embryonic development, including J1s and J2s (Baldwin and Mundo-Ocampo, 1991). A study by Gibson et al. (1995) indicated that mature cysts of *Globodera rostochiensis* could

TABLE 4. Fatty acid profiles for three *Meloidogyne incognita* races, three host plants for *M. incognita* race 3 and *Rotylenchulus reniformis*, and three life stages of *Heterodera glycines*. Means are listed in order by fatty acid chain length, location of the double bond, and functional group.

Fatty Acid	MIR1	MIR2	$MIR3_C$	$MIR3_S$	$MIR3_T$	RR_C	RR_S	RR_T	SCNC	SCNF	SCNV
10:0	0.05	0.08	0.13	0.52	–	1.38	3.72	0.17	–	–	–
10:0 2OH	–†	–	–	1.27	–	0.01	0.10	–	–	–	–
12:0	–	–	–	–	–	–	–	0.11	0.01	–	–
12:0 2OH	2.14	3.55	11.01	8.01	7.86	13.00	20.90	13.12	0.16	–	0.34
13:0	–	–	–	–	–	–	0.02	–	–	–	–
14:0	0.33	–	0.02	0.32	0.14	0.46	0.29	0.20	0.90	7.15	0.81
14:0 2OH	–	–	–	–	0.06	0.34	2.54	0.38	–	–	–
15:0	–	–	–	–	–	0.03	0.74	0.08	–	–	–
15:0 ANTEISO	–	–	–	0.24	–	0.21	0.01	0.36	–	0.54	–
15:0 ISO	0.60	0.38	0.08	0.54	0.46	0.41	0.04	0.50	0.54	3.70	0.49
15:1 ANTEISO A	–	–	–	–	–	–	0.07	–	–	–	–
16:0	7.37	8.74	11.25	33.40	18.46	13.21	19.56	15.87	2.77	16.15	3.64
16:0 2OH	–	–	–	–	0.15	–	0.01	–	–	–	–
16:0 3OH	–	–	–	0.61	–	0.01	–	–	–	–	–
16:0 ANTEISO	–	–	–	–	–	0.41	0.02	0.11	–	–	–
16:1 C	–	–	–	–	–	0.46	0.65	0.04	–	–	–
16:1 CIS 9	–	–	–	–	–	0.36	0.01	0.07	–	–	–
16:1 ω5c	0.44	0.10	–	26.43	–	–	0.41	–	1.48	11.59	1.95
16:1 ω7c	1.18	2.84	0.05	0.48	0.11	–	–	–	1.53	8.87	1.48
17:0	0.04	–	–	–	–	–	0.03	–	–	1.28	–
17:0 ANTEISO	–	–	–	0.12	1.08	2.87	0.23	0.85	–	–	–
17:0 ISO	0.24	–	0.02	0.84	0.08	0.23	0.07	0.16	0.46	0.42	0.45
17:1 ISO I/ANTEI B	–	–	–	–	–	–	–	0.37	–	2.37	–
18:0	14.38	15.09	23.69	7.48	40.25	12.64	15.89	14.42	1.71	4.86	2.89
18:0 3OH	0.05	–	0.01	0.55	–	0.05	0.04	0.13	1.12	0.74	1.26
18:0 ANTE/18:2 ω6,9c	0.33	0.55	–	–	–	0.34	–	0.04	2.16	1.72	–
18:0 ANTEISO/18:2c	–	–	–	–	–	0.19	0.41	0.25	–	–	–
18:1 B	–	–	–	–	–	0.35	0.03	0.13	–	–	–
18:1 CIS 11/t 9/t 6	–	–	–	–	–	14.47	6.24	16.66	–	–	–
18:1 CIS 9	–	–	–	–	–	0.90	0.93	0.73	–	–	–
18:1 TRANS 9/t6/c11	–	–	–	–	–	9.45	12.13	8.43	–	–	–
18:1 ω5c	1.65	0.91	0.05	0.03	0.06	–	–	–	2.31	1.20	2.22
18:1 ω7c	58.94	57.08	46.91	8.34	19.81	1.06	0.98	11.69	60.14	11.64	54.70
18:1 ω9c	2.23	2.18	0.49	5.10	0.14	0.06	0.03	0.17	2.52	5.19	3.39
18:2 CIS 9,12/18:0a	–	–	–	–	–	0.13	0.03	0.40	–	–	–
18:2 ω6,9c/18:0 ANTE	2.60	1.99	1.39	0.81	0.11	0.62	–	0.21	6.01	4.35	6.53
18:3 CIS 6,12,14	–	–	–	–	–	0.02	0.02	0.02	–	–	–
18:3 ω6c (6,9,12)	–	0.21	0.22	0.29	0.26	0.17	1.34	0.53	1.04	–	1.06
19:0	–	–	–	0.18	–	–	–	–	–	–	–
19:0 CYCLO ω8c	–	–	–	1.00	–	–	0.02	–	–	–	–
19:0 ANTEISO	–	–	–	–	–	2.00	–	–	–	–	–
19:1 ISO I	1.35	0.95	0.21	0.07	0.91	0.54	0.11	0.23	0.98	–	1.37
20:0	2.02	1.86	0.98	0.06	1.75	1.66	4.65	3.68	0.52	–	1.37
20:0 ISO	0.02	–	–	–	–	0.03	–	–	0.10	1.87	0.02
20:1 TRANS 11	–	–	–	–	–	2.07	2.26	2.29	–	–	–
20:1 ω7c	3.94	3.50	3.48	0.12	3.00	0.07	0.09	0.91	3.14	–	4.81
20:1 ω9c	–	–	–	–	–	–	–	–	–	–	0.02
20:2 ω6,9c	0.06	–	–	0.03	–	1.38	–	0.06	0.69	4.95	0.98
20:4 CIS 5,8,11,14	–	–	–	–	–	0.18	0.15	0.21	–	–	–
20:4 ω6,9,12,15c	–	–	–	1.57	–	–	–	–	9.63	9.46	10.22
TBSA 10Me18:0	–	–	–	–	–	0.73	–	0.18	0.03	–	–
unknown 10:928	–	–	–	–	–	0.14	0.80	0.24	–	–	–
unknown 15:549	–	–	–	–	–	1.26	1.73	0.93	–	–	–
unknown 16:582	–	–	–	–	0.42	0.60	–	0.37	–	–	–
unknown 18:814	–	–	–	–	8.31	13.18	0.45	4.29	–	–	–
unknown 19:735	–	–	–	–	–	–	0.06	0.05	–	–	–

MIR1: *M. incognita* race 1, MIR2: *M. incognita* race 2, $MIR3_C$: *M. incognita* race 3 increased on cotton, $MIR3_S$: *M. incognita* race 3 increased on soybean, $MIR3_T$: *M. incognita* race 3 increased on tomato, RR_C: *R. reniformis* increased on cotton, RR_S: *R. reniformis* increased on soybean, RR_T: *R. reniformis* increased on tomato, SCNC: *H. glycines* cyst life stage, SCNF: *H. glycines* female life stage, and SCNV: *H. glycines* juvenile life stage.

† = Not Detected.

be identified by the presence of J2s within cysts. A mature *H. glycines* cyst should therefore produce a fatty acid profile comparable to that of *H. glycines* juveniles alone. The fatty acid composition of a cyst itself may be similar to that of females and juveniles, but crushed and emptied cysts would need to be analyzed to determine the actual fatty acid profile of a cyst. Determining fatty acid composition of the cyst may not be necessary

TABLE 5. Phenotypic canonical correlation of fatty acids for the three canonical discriminant functions of FAME profile analysis for 19 nematode variables. 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 $r \geq |0.75|$ (**bold**).

	Response variable	Discriminant variate		
No.	Fatty acid variable	CAN 1	CAN 2	CAN 3
1	10:0	0.251	-0.151	0.633
2	10:0 2OH	0.071	0.692	-0.013
3	12:0	0.084	0.042	-0.131
4	12:0 2OH	0.421	-0.215	0.778
5	13:0	0.075	0.071	-0.170
6	14:0	-0.451	-0.010	-0.082
7	14:0 2OH	0.229	-0.198	0.577
8	15:0	0.199	-0.173	0.550
9	15:0 ANTEISO	0.061	0.101	0.481
10	15:0 ISO	-0.442	-0.055	-0.121
11	15:1 ANTEISO A	0.062	-0.167	0.040
12	16:0	0.347	0.539	-0.239
13	16:0 3OH	0.016	**0.915**	0.219
14	16:0 ANTEISO	0.219	-0.241	0.540
15	16:1 C	0.264	-0.253	0.717
16	16:1 CIS 9	0.205	-0.227	0.511
17	16:1 ω5c	-0.146	**0.893**	0.167
18	16:1 ω7c	0.008	**0.920**	0.199
19	16:1 ω7c/15 iso 2OH	-0.646	-0.132	-0.341
20	17:0	-0.148	0.056	-0.223
21	17:0 ANTEISO	0.267	-0.237	0.518
22	17:0 ISO	-0.334	0.346	0.050
23	17:1 ISO I/ANTEIB	-0.057	0.012	-0.128
24	18:0	0.439	-0.109	-0.292
25	18:0 3OH	-0.624	0.295	-0.017
26	18:0 ANTE/18:2 ω6,9c	-0.011	0.180	-0.294
27	18:0 ANTEISO/18:2 c	0.305	-0.293	**0.766**
28	18:1 CIS 11/t 9/t 6	0.293	-0.305	0.676
29	18:1 TRANS 9/t6/c11	0.321	-0.316	**0.803**
30	18:1 ω5c	-0.716	-0.147	-0.273
31	18:1 ω7c	-0.434	-0.230	-0.478
32	18:1 ω9c	-0.081	0.400	-0.417
33	18:2 CIS 9,12/18:0a	0.216	-0.229	0.445
34	18:2 ω6,9c/18:0 ANTE	-0.630	0.105	-0.323
35	18:3 ω6c (6,9,12)	0.002	0.063	-0.067
36	19:0	0.068	0.145	-0.150
37	19:0 ANTEISO	0.173	-0.189	0.434
38	19:0 CYCLO ω8c	0.012	**0.918**	0.210
39	19:1 ISO I	-0.164	-0.059	-0.359
40	20:0	0.249	-0.251	0.211
41	20:0 ISO	-0.292	0.055	-0.401
42	20:1 ω7c	-0.376	-0.225	-0.484
43	20:1 ω9c	0.063	0.128	-0.293
44	20:4 ω6,9,12,15c	**-0.971**	0.071	0.190
45	unknown 10.928	0.248	-0.226	0.653
46	unknown 15.549	0.315	-0.308	**0.800**
	Eigenvalue	30.84	10.62	8.49
	Cumulative %	45.9	61.7	74.3
	Canonical Correlation	0.98	0.96	0.95

since the Sherlock® Analysis Software is able to differentiate samples containing cysts and juveniles from samples containing only juveniles in all samples studied, indicating that differences in the fatty acid profile due to the cyst has already been accounted for by the software.

The Sherlock® Analysis Software requires a minimum amount of lipids to be present in samples to create a library entry that can reliably identify an unknown sample (Sasser, 1990). Lipid concentrations are the limiting factor to creating library entries, but not to identifying samples. A sample may contain less than the amount of lipids to create a library entry; however, the software will attempt to match that sample to an existing library entry. Generating library entries based on samples containing concentrated lipids allows the system to detect minute fatty acids and produces a more robust fatty acid profile than using dilute samples.

Several aspects of an unknown sample can be determined from the fatty acid profile of that sample. Characteristics such as life stage, host of origin, and race could be determined by the amount of lipid present in a sample. For instance, if a sample of *Meloidogyne* was analyzed, the species and/or race could be identified based on the percentage of 16:0, 18:0, and 18:1 ω9c in the samples. It might be possible to use a fatty acid such as 19:0 cyclo ω8c to determine the host of origin when needed to recommend crop rotations for certain species and races of nematodes. This fatty acid can be present in *Rhizobium* species that induce root nodules in soybean plants (Tighe, 2000). The presence of this fatty acid in a sample may indicate that the nematode contains a specific bacterial species when feeding on *Rhizobium* infested root systems. Of the three hosts studied, only nematodes increased on soybean plants contained the 19:0 cyclo ω8c fatty acid.

Many of the diagnostic laboratories in our area identify plant parasitic nematodes to the genus level which is appropriate for recommendation of nematicide applications. However, this might not be adequate in other parts of the country. Furthermore, the species and races of *Meloidogyne* samples must be determined to make the proper crop rotation recommendations because of the host specificity exhibited by different species and races. The library developed herein can identify these genera of nematodes with 99% accuracy, and only decreases to 97% for identifications to the species level. Diagnostic laboratories utilizing this software could identify species or races within *Meloidogyne* with at least 87% accuracy. Identification of nematode genera, species, races, and life stages with this system can reduce the total work required for sample processing in diagnostic laboratories.

Based on the success of this research, it is possible to use FAME analysis and the Sherlock® Analysis Software as an alternative means for identification of plant parasitic nematodes in diagnostic laboratories. Using this approach would reduce the time required for identifications to the species and race levels, which can take up to 45 days to complete for *Meloidogyne* species and races (Taylor and Sasser, 1978). It may even be possible to detect and identify plant parasitic nematodes directly from extracted soil samples based on comparative studies of soil FAME profiles. Studies by Madan (2002) and Ruess et al. (2002) have indicated that FAME analysis

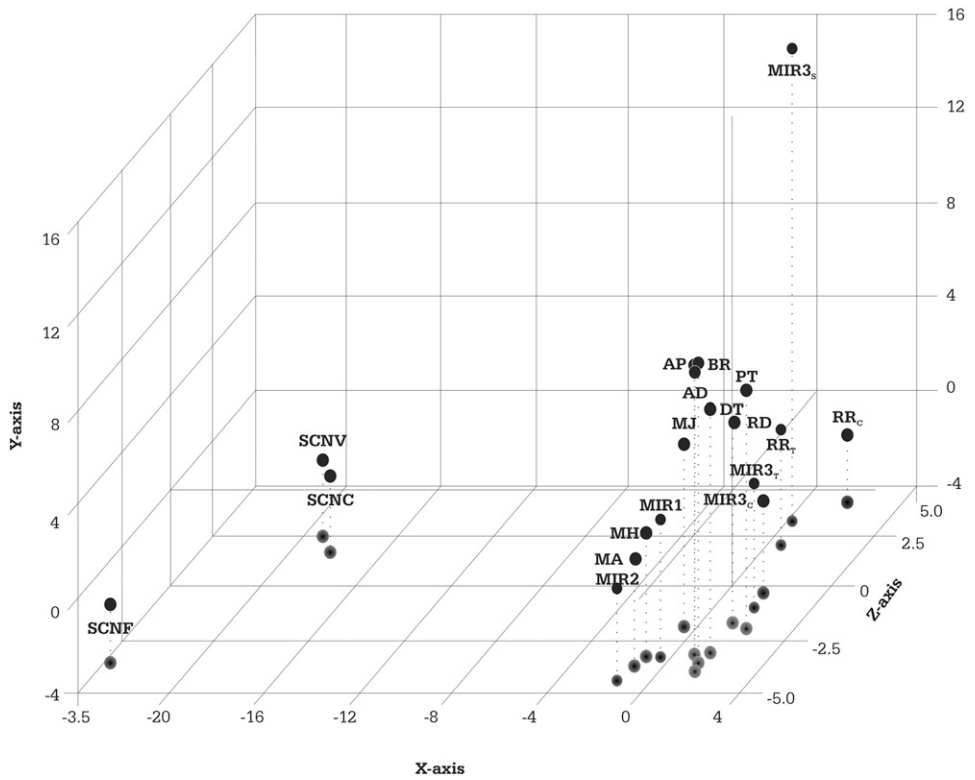

FIG. 2. Graph of canonical means for twelve nematode species, including three life stages of *Heterodera glycines*, three host plants for *Rotylenchulus reniformis* and *Meloidogyne incognita*, and three races of *M. incognita*. Placement is defined for the first (x-axis), second (y-axis), and third (z-axis) canonical axes by height (y-axis) and shadow (x-axis and z-axis intercept). AD: *Aphelenchoides fragariae*, AP: *Aphelenchus avenae*, BR: *Bursaphelenchus xylophilus*, DT: *Ditylenchus dipsaci*, MA: *Meloidogyne arenaria*, MH: *M. hapla*, MIR1: *M. incognita* race 1, MIR2: *M. incognita* race 2, MIR3C: *M. incognita* race 3 increased on cotton, MIR3S: *M. incognita* race 3 increased on soybean, MIR3T: *M. incognita* race 3 increased on tomato, MJ: *M. javanica*, PT: *Pratylenchus penetrans*, RD: *Radopholus similis*, RRC: *Rotylenchulus reniformis* increased on cotton, RRS: *Rotylenchulus reniformis* increased on soybean, RRT: *Rotylenchulus reniformis* increased on tomato, SCNC: *Heterodera glycines* cyst life stage, SCNF: *Heterodera glycines* female life stage, and SCNV: *Heterodera glycines* juvenile life stage.

could be used to detect, and possibly quantify, fungal and nematode species in soil extractions. In addition to identification, information found in the development of this library could have potential to be valuable in other areas of agriculture, such as using fatty acids as a means for resistance induction in crop plants. Zinovieva et al. (1995) observed that treating tomato seeds with varying concentrations of the 20:4 ω6,9,12,15c fatty acid reduced *M. incognita* numbers and increased the production of nematotoxic compounds by treated plants.

TABLE 6. Comparison matrix for 13 nematode fatty acid profiles generated using the Sherlock® Analysis Software. Comparisons are listed by column with numbers indicating the percentage of samples from each column matching to the indicated row.

	SCNV	SCNC	SCNF	MA	MH	MIR1	MIR2	$MIR3_C$	$MIR3_T$	MJ	RR_C	RR_S	RR_T
SCNV	100.0	.	.	.	.	.	.	.	.	.	.	.	.
SCNC	.	100.0	.	.	.	.	.	.	.	.	.	.	.
SCNF	.	.	100.0	.	.	.	.	.	.	.	.	.	.
MA	.	.	.	77.8	.	5.0	1.3	.	.	.	.	.	.
MH	.	.	.	16.7	94.4	24.4	6.4	.	.	.	.	.	.
MIR1	.	.	.	.	.	64.7	7.7	3.0	.	.	.	.	.
MIR2	.	.	.	.	.	5.9	76.9	.	.	.	.	.	.
$MIR3_C$	.	.	.	.	.	.	7.7	97.0	.	.	.	.	.
$MIR3_T$	.	.	.	.	.	.	.	.	100.0	.	.	.	.
MJ	.	.	.	.	.	.	.	.	.	100.0	.	.	.
RR_C	.	.	.	5.6	5.6	.	.	.	.	.	94.0	.	13.6
RR_S	.	.	.	.	.	.	.	.	.	.	2.0	95.0	.
RR_T	.	.	.	.		.	.	.	.	.	4.0	5.0	86.4

MA: *Meloidogyne arenaria*, MH: *M. hapla*, MIR1: *M. incognita* race 1, MIR2: *M. incognita* race 2, $MIR3_C$: *M. incognita* race 3 increased on cotton, $MIR3_T$: *M. incognita* race 3 increased on tomato, MJ: *M. javanica*, RR_C: *Rotylenchulus reniformis* increased on cotton, RR_S: *Rotylenchulus reniformis* increased on soybean, RR_T: *Rotylenchulus reniformis* increased on tomato, SCNC: *Heterodera glycines* cyst life stage, SCNF: *Heterodera glycines* female life stage, and SCNV: *Heterodera glycines* juvenile life stage.

Many disciplines have the potential to gain from using FAME analysis as a means for identification of plant parasitic nematodes.

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