FAME ANALYSIS-ESPIED POPULATIONS OF *ROTYLENCHULUS RENIFORMIS* IN SOIL

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Abstract

Previous research has indicated that it may be possible to identify or detect organisms, such as bacteria or fungi, found in soil using FAME analysis. Our objective is to develop a FAME profile of *Rotylenchulus reniformis* to detect the plant-parasitic nematode in soil samples. Forty pots of cotton were grown for sixty days under greenhouse conditions. Three 1.0 g-samples were taken from each pot of two groups, those that were grown in the absence of *R. reniformis*, and the other inoculated with 2000 *R. reniformis* individuals per 500 cm^3 for the growing period. Each of the 120 samples was extracted and analyzed using FAME gas chromatography. The resulting fatty acid profiles for all samples were analyzed using the STEPDISC and CANDISC procedures of SAS (SAS Institute, Inc). Sixty-four fatty acids total were detected. Of these, six were found to be significant for differentiating between samples containing or lacking *R. reniformis*. The total Mahalanobis distance (D^2) between the soil samples with *R. reniformis* and without was 13.67 ($P \leq 0.0001$). The six significant fatty acids varied in their expression between inoculated and control soil types. Of these fatty acids, 12:0 2OH appeared to be specific for the presence of *R. reniformis*. The 12:0 2OH fatty acid was found at a concentration of less than 0.04% in soil samples lacking *R. reniformis*. In samples containing *R. reniformis*, the mean sample percentage was 1.27%. Since this is a fatty acid found in the FAME profile of *R. reniformis*, it may be possible to use the presence of 12:0 2OH as an indicator of *R. reniformis* in soil samples.

Introduction

Previous research has indicated that fungi can be isolated from soil samples using fatty acid methyl ester (FAME) analysis (Graham *et al*., 1995; Madan *et al.*, 2002). These studies generated known FAME profiles for species of fungi that form mycorrhizal relationships with many land plants and compared those profiles to soil extractions with and without the fungi present. By using FAME analysis, they were able to detect the fungi within the soil samples. Ruess (2002) also indicated that the fatty acid profiles generated from analyzing soil samples with fungi or nematodes could be used to indicate the presence of those organisms.

Our previous research has developed a FAME profile for *Rotylenchulus reniformis* and has shown that this nematode can be detected and identified in populations greater than 250 individuals in pure culture (Sekora *et al*. 2008). It is hypothesized that the FAME profile for *R. reniformis* can be used to detect the nematode in soil samples generated under controlled conditions.

Materials and Methods

Experimental design for the trial consisted of two treatments with twenty replications each. Treatment one contained twenty pots of cotton not inoculated with *R. reniformis*. Treatment two was composed of twenty pots of cotton infested with 2000 individuals of *R. reniformis* each. Both treatments were spaced to prevent splashing among pots and allowed to grow for 60 days in the greenhouse (Figure 1).

Figure 1. Potting arrangement for samples

From each pot, three one-gram samples of soil were analyzed using GC-FAME. Treatments were analyzed for differences in fatty acid profiles and the presence of known *R. reniformis* fatty acids using the Sherlock Analysis Software (MIDI, Inc.). Statistical analysis by the STEPDISC and CANDISC procedures of SAS (SAS Institute, Inc.) was used to differentiate treatments based on fatty acids present.

Results and Discussion

Soil containing *R. reniformis* had a statistically different FAME profile than soil absent of *R. reniformis* (D^2= 13.67, $P \leq 0.0001$). Six of sixty-four fatty acids were significant for distinguishing between the inoculated and control soil types, though there was variation among all fatty acids (Figure 2, Table 1.).

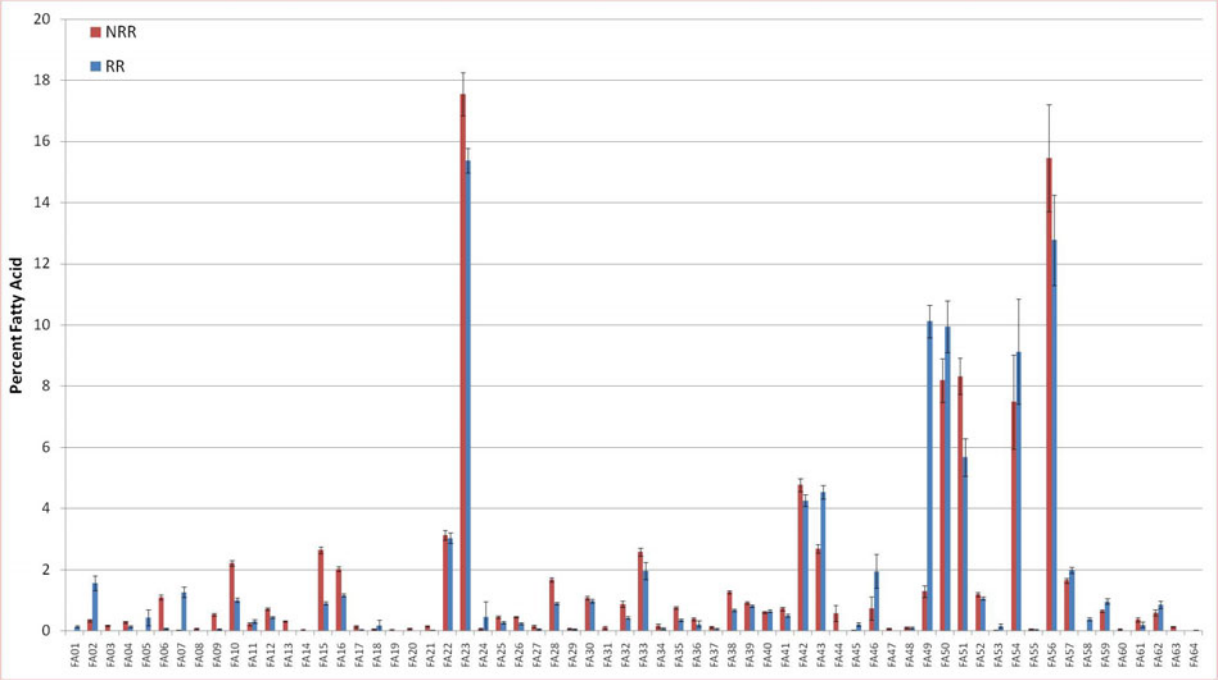

Figure 2. Fatty acid profiles of soil samples with and without *Rotylenchulus reniformis* present.

Table 1. Fatty acid abbreviation key for Figure 2.

Name	Fatty Acid
FA01	10:0
FA02	10:0 2OH
FA03	10:0 3OH
FA04	11 methyl 18:1 w7c
FA05	11:0 ANTEISO
FA06	12:0
FA07	12:0 2OH
FA08	12:0 3OH
FA09	13:0 ANTEISO
FA10	14:0
FA11	14:0 2OH
FA12	14:0 3OH/16:1 ISO I
FA13	14:0 ISO
FA14	15:0 3OH
FA15	15:0 ANTEISO
FA16	15:0 ISO
FA17	15:0 ISO 2OH/16:1w7c
FA18	15:0 ISO 3OH
FA19	15:1 ANTEISO A
FA20	15:1 ISO G
FA21	15:1 w6c
FA22	15:1 w8c

Name	Fatty Acid
FA23	16:0
FA24	16:0 10 methyl
FA25	16:0 2OH
FA26	16:0 3OH
FA27	16:0 ANTEISO
FA28	16:0 ISO
FA29	16:0 ISO 3OH
FA30	16:0 N alcohol
FA31	16:1 ISO I/14:0 3OH
FA32	16:1 w5c
FA33	16:1 w7c/15 iso 2OH
FA34	16:1 w9c
FA35	17:0
FA36	17:0 10 methyl
FA37	17:0 2OH
FA38	17:0 ANTEISO
FA39	17:0 CYCLO
FA40	17:0 ISO
FA41	17:0 ISO 3OH
FA42	17:1 w7c
FA43	18:0
FA44	18:0 2OH

Name	Fatty Acid
FA45	18:0 3OH
FA46	18:0 ANTE/18:2 w6,9c
FA47	18:0 ISO
FA48	18:1 2OH
FA49	18:1 w7c
FA50	18:1 w9c
FA51	18:2 w6,9c/18:0 ANTE
FA52	18:3 w6c (6,9,12)
FA53	19:0
FA54	19:0 CYCLO w10c/19w6
FA55	19:0 ISO
FA56	19:1 w6c/.846/19cy
FA57	20:0
FA58	20:1 w7c
FA59	20:4 w6,9,12,15c
FA60	ANTEISO 17:1 w9c
FA61	ISO 17:1 w10c
FA62	ISO 17:1 w9c
FA63	TBSA 10Me18:0
FA64	unknown 14.263

Of the six significant fatty acids, 12:0 2OH is a fatty acid found in *R. reniformis*. The mean concentration of this fatty acid is less than 0.04% in samples devoid of *R. reniformis*, but 1.27% in samples with *R. reniformis* within the soil. The remaining samples are not found in the FAME profile of *R. reniformis*. The fatty acid 10:0 was found at a mean concentration of 0.2% in *R. reniformis* soil samples, but was absent in non-inoculated soil. The fatty acids 12:0, 12:0 Anteiso, and 14:0 Iso were present at 1.1%, 0.5%, and 0.3% respectively within soil samples without *R. reniformis* compared to 0.08, 0.05, and 0.0% in samples with *R. reniformis* (Figure 3).

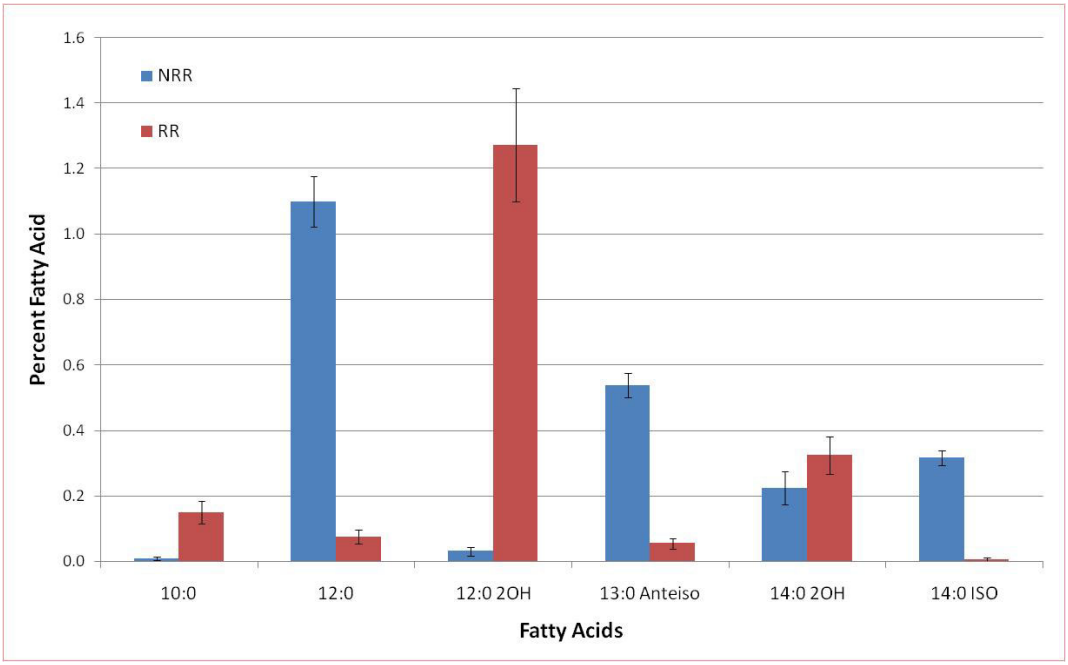

Figure 3. Mean percentages of fatty acids significant for differentiation between soil types. Error bars indicate differences at $P \leq 0.05$).

Conclusions

Even though the majority of the fatty acids observed from the soil analysis are not significant for identification, those that are may be crucial to indicating differences in soil populations of other nematodes, bacteria, fungi, and other soil organisms based on the presence or absence of *R. reniformis*. Soil properties such as texture, color, and ecology are visibly different in soils lacking *R. reniformis* than those of soil containing the nematode. The differences observed in the fatty acid profiles of soil samples with and without *R. reniformis* may be consistent enough to indicate the presence of this nematode in the soil sample. Previous research has also indicated that the mean concentration of 12:0 2OH in samples containing 5000 individuals of *R. reniformis* is 1.42%. (Sekora *et al*., 2009) It may be possible to use the 12:0 2OH fatty acid as an indicator of *R. reniformis* in soil samples analyzed by FAME analysis and could possibly indicate population numbers based on percentage.

References

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