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 ($P<0.0001$). 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).

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* ($D^2= 13.67, P<0.0001$). 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Fatty Acid</th>
<th>Name</th>
<th>Fatty Acid</th>
<th>Name</th>
<th>Fatty Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA01</td>
<td>10:0</td>
<td>FA23</td>
<td>16:0</td>
<td>FA45</td>
<td>18:0 3OH</td>
</tr>
<tr>
<td>FA02</td>
<td>10:0 2OH</td>
<td>FA24</td>
<td>16:0 10 methyl</td>
<td>FA46</td>
<td>18:0 ANTE/18:2 w6,9c</td>
</tr>
<tr>
<td>FA03</td>
<td>10:0 3OH</td>
<td>FA25</td>
<td>16:0 2OH</td>
<td>FA47</td>
<td>18:0 ISO</td>
</tr>
<tr>
<td>FA04</td>
<td>11 methyl 18:1 w7c</td>
<td>FA26</td>
<td>16:0 3OH</td>
<td>FA48</td>
<td>18:1 2OH</td>
</tr>
<tr>
<td>FA05</td>
<td>11:0 ANTEISO</td>
<td>FA27</td>
<td>16:0 ANTEISO</td>
<td>FA49</td>
<td>18:1 w7c</td>
</tr>
<tr>
<td>FA06</td>
<td>12:0</td>
<td>FA28</td>
<td>16:0 ISO</td>
<td>FA50</td>
<td>18:1 w9c</td>
</tr>
<tr>
<td>FA07</td>
<td>12:0 2OH</td>
<td>FA29</td>
<td>16:0 ISO 3OH</td>
<td>FA51</td>
<td>18:2 w6,9c/18:0 ANTE</td>
</tr>
<tr>
<td>FA08</td>
<td>12:0 3OH</td>
<td>FA30</td>
<td>16:0 N alcohol</td>
<td>FA52</td>
<td>18:3 w6c (6,9,12)</td>
</tr>
<tr>
<td>FA09</td>
<td>13:0 ANTEISO</td>
<td>FA31</td>
<td>16:1 ISO I/14:0 3OH</td>
<td>FA53</td>
<td>19:0</td>
</tr>
<tr>
<td>FA10</td>
<td>14:0</td>
<td>FA32</td>
<td>16:1 w5c</td>
<td>FA54</td>
<td>19:0 CYCLO w10c/19w6</td>
</tr>
<tr>
<td>FA11</td>
<td>14:0 2OH</td>
<td>FA33</td>
<td>16:1 w7c/15 iso 2OH</td>
<td>FA55</td>
<td>19:0 ISO</td>
</tr>
<tr>
<td>FA12</td>
<td>14:0 3OH/16:1 ISO I</td>
<td>FA34</td>
<td>16:1 w9c</td>
<td>FA56</td>
<td>19:1 w6c/.846/19cy</td>
</tr>
<tr>
<td>FA13</td>
<td>14:0 ISO</td>
<td>FA35</td>
<td>17:0</td>
<td>FA57</td>
<td>20:0</td>
</tr>
<tr>
<td>FA14</td>
<td>15:0 3OH</td>
<td>FA36</td>
<td>17:0 10 methyl</td>
<td>FA58</td>
<td>20:1 w7c</td>
</tr>
<tr>
<td>FA15</td>
<td>15:0 ANTEISO</td>
<td>FA37</td>
<td>17:0 2OH</td>
<td>FA59</td>
<td>20:4 w6,9,12,15c</td>
</tr>
<tr>
<td>FA16</td>
<td>15:0 ISO</td>
<td>FA38</td>
<td>17:0 ANTEISO</td>
<td>FA60</td>
<td>ANTEISO 17:1 w9c</td>
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<td>FA17</td>
<td>15:0 ISO 2OH/16:1w7c</td>
<td>FA39</td>
<td>17:0 CYCLO</td>
<td>FA61</td>
<td>ISO 17:1 w10c</td>
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<tr>
<td>FA18</td>
<td>15:0 ISO 3OH</td>
<td>FA40</td>
<td>17:0 ISO</td>
<td>FA62</td>
<td>ISO 17:1 w9c</td>
</tr>
<tr>
<td>FA19</td>
<td>15:1 ANTEISO A</td>
<td>FA41</td>
<td>17:0 ISO 3OH</td>
<td>FA63</td>
<td>TBSA 10Me18:0</td>
</tr>
<tr>
<td>FA20</td>
<td>15:1 ISO G</td>
<td>FA42</td>
<td>17:1 w7c</td>
<td>FA64</td>
<td>unknown 14.263</td>
</tr>
<tr>
<td>FA21</td>
<td>15:1 w6c</td>
<td>FA43</td>
<td>18:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA22</td>
<td>15:1 w8c</td>
<td>FA44</td>
<td>18:0 2OH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 $P \leq 0.05$.](image)

**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


