Effect of Short-Chain Fatty Acids and Soil Atmosphere on Tylenchorhynchus spp.¹

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Abstract: Short-chain fatty acids can be produced under anaerobic conditions by fermentative soil microbes and have nematicidal properties. We evaluated the effects of butyric and propionic acids on death and recovery of stunt nematodes (Tylenchorhynchus spp.), a common parasite of turfgrass. Nematodes in a sand-soil mix (80:20) were treated with butyric or propionic acid and incubated under air or N₂ for 7 days at 25 °C. Amendment of soil with 0.1 and 1.0 µmol (8.8 and 88 µg) butyric acid/g soil or 1.0 µmol (74 µg) propionic acid/g soil resulted in the death of all nematodes. The composition of the soil atmosphere had no effect on the nematicidal activity of the acids. Addition of hydrochloric acid to adjust soil pH to 4.4 and 3.5 resulted in nematode mortality relative to controls (41% to 86%) but to a lesser degree than short-chain fatty acids at the same pH. Nematodes did not recover after a 28-day period following addition of 10 µmol butyric acid/g soil under air or N₂. Carbon mineralization decreased during this period, whereas levels of inorganic N and microbial biomass-N remained constant. Short-chain fatty acids appear to be effective in killing Tylenchorhynchus spp., independent of atmospheric composition. Nematode mortality appears to be a function of the type and concentration of fatty acid and soil pH.

Key words: butyric acid, carbon mineralization, fatty acid, nematicide, nematodes, propionic acid, Tylenchorhynchus spp.

Nematode parasitism of turfgrass roots impairs ability to absorb water and nutrients, resulting in disease symptoms that include wilting, chlorosis, and stunting. Excessive root branching, galling, and necrosis may occur at feeding sites. The sandy root zone mix employed in golf putting greens construction is conducive to plant-parasitic nematode establishment and population increase (Pérez and Lewis, 2001).

Synthetic nematicides provide short-term control of plant-parasitic nematodes but populations may rebound, and there is a potential for the development of resistant biotypes (Jatala, 1986). These chemicals have proved expensive, hazardous to apply, and persistent and may contaminate groundwater (Jatala, 1986). Fenamiphos, a systemic organophosphate that acts as a nematostatin, remains available for use on golf course greens in some areas, but there have been reports of reduced efficacy due to rapid microbial degradation (Stirling et al., 1992). Recently enacted government regulations (Food Quality Protection Act [FQPA]) direct the manufacturer to cease the production of fenamiphos by 2007 (Crow, 2002). The fumigants methyl bromide and dazomet, which have been used to eliminate nematodes during complete greens renovation (Pérez and Lewis, 2001), provide short-term control. Metam sodium and 1,3-dichloropropene have been approved for limited use on fairways, roughs, driving ranges, and tees in Florida for nematode control (Crow, 2002), and 1,3-dichloropropene is also approved for use in golf courses in South Carolina, Georgia, and Alabama (Dow AgroSciences, 2004). However, their use requires specialized equipment and sophisticated application techniques.

Concerns about environmental risks associated with synthetic nematicides have led researchers to look for alternatives. A wide variety of organic amendments have been tested (Browning et al., 1999; Feder, 1960; Muller and Gooch, 1982; Rodriguez-Kabana and Morgan-Jones, 1987), mainly in crop soils, in an effort to suppress plant-parasitic nematodes. In most instances their mode of action is not well understood. Mechanisms implicating toxic levels of ammonia or other chemicals present or generated in amendments by microorganisms have been proposed. Proliferation of antagonistic organisms has also been suggested.

Rodriguez-Kabana (1965) reported development of nematode-suppressive soils in rice paddies due to flooding. Johnston (1959a) attributed a reduction in Tylenchorhynchus maritini (now T. annulatus) in saturated soil to the activity of the anaerobic bacterium Clostridium butyricum. Of the fermentation products identified from the activities of C. butyricum, only the short-chain fatty acids— butyric, propionic, acetic, and formic acids—exhibited nematicidal properties. Because short-chain fatty acids are also found in aerobic soils (Küsel and Drake, 1999; Rodríguez-Kabana, 1965), they may provide a safe alternative to synthetic nematicides because they occur naturally in soil and are readily oxidized to carbon dioxide and water by soil microorganisms. The development of alternative agents for control of plant-parasitic nematodes may also impact pest management in crops other than turfgrass because the availability of some synthetic nematicides used in other crop protection applications is expected to become restricted in response to FQPA regulations.

We conducted a series of experiments to evaluate the effectiveness of two short-chain fatty acids—butyric and propionic acids—in controlling plant-parasitic nematodes. Our objectives were to (i) evaluate the effects of butyric and propionic acids on Tylenchorhynchus spp.
under both aerobic and anaerobic soil atmospheres, (ii) distinguish between the effects of pH and the fatty acids themselves, and (iii) evaluate the potential for recovery of nematode and microbial communities following exposure to nematicidal levels of butyric acid. We postulated the nematicidal activity of fatty acids to be concentration dependent and to result from factors other than acidification of the soil. In addition, we expected increased efficacy in an anaerobic soil atmosphere due to reduced mineralization of fatty acids.

Materials and Methods

Source of test organisms: Nematode-infested soil, dominated by Tylenchorhynchus spp., was acquired from disease sample cores submitted to the University of Rhode Island’s Turf Diagnostic Clinic from golf courses throughout New England. Nematodes were extracted with Baermann trays and allowed to reproduce on creeping bentgrass (Agrostis palustris cv Penncross) plants grown in a greenhouse. After 6 months the plants were discarded and the nematode-infested soil was combined at a 1:4 ratio with autoclaved 80:20 (sand:soil) mix. The resulting soil had a pH of 5.2 and was stored in the dark at 4 °C.

Effects of fatty acids and atmospheric composition on Tylenchorhynchus spp.: Treatments consisted of soil amended with different concentrations of butyric or propionic acid and maintained under aerobic or anaerobic conditions. Butyric acid was purchased from Kodak Laboratory Chemicals (Rochester, NY), and propionic acid was purchased from Mallinkrodt Laboratory Chemicals (Phillipsburg, NJ). Distilled water was used for control treatments. Ten grams fresh weight of nematode-infested soil was placed in a 20-ml glass serum vial and fatty acid solutions (1 ml) added to yield 10 µmol butyric acid/g soil, with control treatments receiving 1 ml distilled water. The headspace gases in half of the vials was replaced with nitrogen gas. Following initial incubation in the dark for 7 days at 25 °C, the headspace in all vials was replaced with air. Microbial activity and nematode populations were monitored over 28 days by evaluating soil pH, nematode density, C mineralization, soil inorganic nitrogen, and microbial biomass N every 7 days.

Each treatment (butyric acid × atmospheric composition) was replicated 14 times within an incubation period. All 14 samples were analyzed for CO₂ evolution every 7 days. Vials were subsequently sampled for nematode density (n = 6), microbial biomass N (n = 4), and soil pH and inorganic N (n = 4).

Levels of inorganic N (NH₄⁺ and NO₃⁻) were determined using the KCl extraction method (Keeney and Nelson, 1982). Analysis was performed with an Alpkem Rapid Flow Analyzer (RFA-300, OI Analytical, College Station, TX). Microbial biomass N was determined by the fumigation-extraction method (Vance et al., 1987). Potassium sulfate extracts of both fumigated and unfumigated samples were oxidized using the alkaline persulfate oxidation method (Cabrera and Beare, 1993). Microbial biomass N was calculated by subtracting N in the unfumigated soil extract from N in the fumigated extract. A conversion factor was not used because of the wide variability of values found in the literature (Van Veen et al., 1985).

Statistical analysis: Normality of data sets was determined using the Kolmogorov-Smirnov test. Depending on the normality of the data, either a Mann-Whitney rank sum test or a t-test was used to test the null hypothesis that two treatments were not drawn from populations with different means or medians.

Data sets that were not normally distributed and included more than two treatments were analyzed using a Kruskal-Wallis analysis of variance on ranks to test the null hypothesis that there was no difference among the population medians. Multiple comparisons vs. a control
were conducted using Dunn’s test to compare treatment groups to the control. For normally distributed data sets including more than two treatments, a one-way analysis of variance was conducted, and multiple comparisons vs. a control were conducted using the Bonferroni t-test. All tests were evaluated at the 95% confidence level (P ≤ 0.05) (SigmaStat for Windows, vers. 2.03, SPSS Inc., Chicago, IL).

**Results**

*Effects of fatty acids on Tylenchorhynchus spp., pH, and C mineralization: * Butyric acid was equally effective in killing all *Tylenchorhynchus* spp. present in soil when added at 0.1 and 1.0 µmol/g soil, regardless of soil atmospheric composition (Table 1). By contrast, addition of 0.01 µmol butyric acid/g soil had no impact on nematode survival relative to the control treatment. Addition of 1 µmol butyric acid g soil \(^{-1}\) lowered soil pH under both aerobic and anaerobic conditions relative to the control (P ≤ 0.05) (Table 1).

Propionic acid was less effective than butyric acid, with a reduction in the number of nematodes observed only at 1.0 µmol/g soil (P ≤ 0.05) (Table 1). As with butyric acid, soil atmosphere had no impact on the nematicidal activity of propionic acid. Soil pH was lower than untreated controls following addition of propionic acid at 1 µmol/g soil under aerobic conditions (P ≤ 0.05) (Table 1).

The cumulative amount of CO\(_2\) evolved in soil treated with 0, 0.01, 0.1, and 1.0 µmol butyric acid/g soil, and incubated in aerobic or anaerobic soil atmospheres for 7 days is shown in Figure 1. Under aerobic conditions, microbial respiration in soil treated with 0.1 and 1.0 µmol butyric acid/g soil (0.25 and 0.37 µmol CO\(_2\)/g soil/day, respectively) decreased relative to the control treatment (0.77 µmol CO\(_2\)/g soil/day) (P ≤ 0.05). Under anaerobic conditions, respiration was not reduced in any butyric acid treatment relative to the control. In contrast, at the lowest level of butyric acid (0.01 µmol/g soil) the amount of CO\(_2\) evolved was higher than in the control under aerobic conditions (P ≤ 0.05). Propionic acid inhibited soil respiration under both aerobic and anaerobic atmospheres only at the highest application rate, 1.0 µmol/g soil (P ≤ 0.05) (0.15 and 0.27 µmol CO\(_2\)/g soil/day, respectively) compared to 0.66 and 0.35 µmol CO\(_2\)/g soil/day, respectively, in the controls.

*Effects of inorganic acid (HCl) on nematode survival and carbon mineralization: * The addition of 0.25 and 1.75 µmol HCl/g reduced soil pH from an initial value of 4.9 to 4.4 and 3.5, respectively (Table 2). Nematode densities declined after incubation for 7 days in acidified soil by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2). Under anaerobic conditions, soil pH was reduced from 4.9 to 4.4 and 3.5, with nematode densities declined by 42% at pH 4.3 and 68% at pH 3.5 relative to the control (Table 2).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Amount added (µmol/g)</th>
<th>Aerobic Nematodes/10 g</th>
<th>Soil pH</th>
<th>Anaerobic Nematodes/10 g</th>
<th>Soil pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric</td>
<td>0.00</td>
<td>80 ± 54</td>
<td>5.3</td>
<td>90 ± 29</td>
<td>5.3</td>
</tr>
<tr>
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<td>0.01</td>
<td>90 ± 63</td>
<td>3.9</td>
<td>100 ± 63</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0 ± 0*</td>
<td>3.6</td>
<td>0 ± 0*</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0 ± 0*</td>
<td>3.1*</td>
<td>0 ± 0*</td>
<td>3.1*</td>
</tr>
<tr>
<td>Propionic</td>
<td>0.00</td>
<td>120 ± 37</td>
<td>3.3</td>
<td>90 ± 84</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>120 ± 37</td>
<td>4.6</td>
<td>40 ± 25</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>120 ± 84</td>
<td>4.0</td>
<td>110 ± 42</td>
<td>4.2*</td>
</tr>
<tr>
<td></td>
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<td>0 ± 0*</td>
<td>3.0*</td>
<td>0 ± 0*</td>
<td>3.0*</td>
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</tbody>
</table>

Values followed by (*) within a column and fatty acid were significantly different from the control (P ≤ 0.05).

<table>
<thead>
<tr>
<th>Amount of acid added (µmol/g)</th>
<th>Aerobic Nematodes/10 g</th>
<th>Soil pH</th>
<th>Anaerobic Nematodes/10 g</th>
<th>Soil pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>65 ± 18</td>
<td>4.9</td>
<td>90 ± 30</td>
<td>4.9</td>
</tr>
<tr>
<td>0.25</td>
<td>37 ± 18*</td>
<td>4.4*</td>
<td>45 ± 25*</td>
<td>4.4</td>
</tr>
<tr>
<td>1.75</td>
<td>20 ± 12*</td>
<td>3.5*</td>
<td>15 ± 19*</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Values followed by (*) within a column were significantly different from the control (P ≤ 0.05).
densities declining by 52% at pH 4.4 and 86% at pH 3.5 relative to the control.

The rate of CO$_2$ evolution declined relative to the control in acidified soil under both aerobic and anaerobic conditions (\(P \leq 0.05\)) (Fig. 2). Carbon mineralization rates in aerobic soil were 0.18 and 0.27 µmol CO$_2$/g soil/day at soil pH of 4.3 and 3.5, respectively, compared to 0.69 µmol CO$_2$/g soil/day in the controls (pH 4.9). Under anaerobic conditions C mineralization rates were reduced from 0.57 µmol CO$_2$/g soil/day in the controls (pH 4.9) to 0.23 and 0.28 µmol CO$_2$/g soil/day at soil pH of 4.4 and 3.5, respectively.

Recovery of *Tylenchorhynchus* spp. and the microbial activity following treatment with butyric acid: We selected butyric acid to study the effects of short-chain fatty acids on recovery of nematodes and of the microbial activity because it was more effective at reducing nematode numbers per g soil at a lower concentration than propionic acid. We used a higher concentration of butyric acid (10 µmol g soil$^{-1}$) than used in previous experiments to study recovery of nematodes and microbial activity under severe conditions. No live nematodes were present in treated samples held under aerobic or anaerobic conditions 7 days after treatment (Fig. 3), and no recovery of nematode populations was observed over the following 21 days regardless of headspace composition. Soil pH was lower in treated soils compared to the controls on all sampling dates (\(P \leq 0.05\)). Under aerobic conditions, the pH of treated soil was initially 3.4, rising to 3.7 after 14 days following treatment, a value maintained throughout the remainder of the experiment. The pH range in the aerobic control treatment ranged from 4.1 to 4.7 over the same period. Soil pH was less consistent under anaerobic conditions, fluctuating from 3.5 to 4.0 in soil amendment with butyric acid and from 4.3 to 4.7 in the control treatment.

The C mineralization rate of soil amended with butyric acid (10 µmol/g soil) decreased under both aerobic and anaerobic conditions relative to the control on all sampling dates (\(P \leq 0.05\)) (Fig. 4) and was undetectable 21 days after treatment with butyric acid.

We did not observe differences between soil treated with 10 µmol butyric acid/g soil and untreated soil in terms of inorganic N, NO$_3^-$, or NH$_4^+$ levels after incubation for 28 days, regardless of headspace composition (data not shown). Similarly, microbial biomass-N was not affected by treatment with butyric acid after incubation for 28 days (data not shown) under aerobic or anaerobic conditions.

**DISCUSSION**

Our results support the hypothesis that short-chain fatty acids have nematicidal properties. Earlier studies involved the production of organic acids by anaerobic bacteria in saturated soils (Browning et al., 1999; Hollis and Rodriguez-Kabana, 1966; Johnston, 1959a) or immersing nematodes in an aqueous acid solution (Bange and Visser, 1965; Dijan et al., 1994; Johnston, 1959b; Stephenson, 1945; Tarjan, 1956). Our study demonstrated that both butyric and propionic acids reduced *Tylenchorhynchus* spp. numbers in unsaturated soil under both aerobic and anaerobic conditions.

We found that butyric acid was more effective than propionic acid in controlling *Tylenchorhynchus* spp. Similarly Johnston (1959b), who exposed *T. annulatus*
to a series of fatty acid solutions, ranked the acids in order of toxicity as follows: butyric > propionic > acetic > formic, a series that correlates with molecular weight of the fatty acid. By contrast, Banage and Visser (1965) reported little difference among fatty acid solutions in toxicity to *Dorylaimus* sp. They hypothesized that as the ionization constants for the fatty acids are similar—resulting in roughly the same fraction of undissociated molecules at the same pH—permeation rates through the nematode cuticle should be similar. The methods employed by Johnston (1959b) and Banage and Visser (1965) involved a short immersion period in organic acid solution followed by a recovery period in tap water. Johnston (1959b) reported an inactivation time for *T. annulatus* in 0.01 M butyric acid (aq) to be approximately 2.5 minutes compared to 5.2 minutes reported for *Dorylaimus* (Banage and Visser, 1965). Differences in test organisms may account for the reported lack of differential toxicity among acids observed by Banage and Visser (1965). The *Dorylaimus* spp., an omnivore/predator, studied by Banage and Visser (1965) is considerably larger than most *Tylenchorhynchus* spp. Browning et al. (2004) reported that bacterivorous, fungivorous, and entomophagous nematodes followed the expected trend of increasing surface area to volume ratios, resulting in an increased surface area through which protonated forms of butyric acid can diffuse. This resulted in a lower LD$_{50}$ with increasing surface-to-volume ratio. In contrast, as the surface-to-volume ratio of plant-parasitic nematodes increased, the LD$_{50}$ for butyric acid increased. Browning et al. (2004) also found differential sensitivity of nematodes to butyric acid based on trophic group, with plant-parasitic nematodes more sensitive than free-living nematodes. Dijan et al. (1994) reported differential sensitivity based on trophic groups from a nematode immersion study in maleic and pentanoic acid solutions.

Addition of 0.1 µmol butyric acid/g soil (8.8 µg/g) resulted in the death of 100% of *Tylenchorhynchus* spp. in 7 days in a sand/soil mixture. The lowest effective rate of butyric acid for suppression of *T. claytoni* in sand over 7 days was reported by Browning et al. (2004) as 880 µg/g, which resulted in a 99% reduction in nematode numbers. Differences in results between sand and sand/soil mixtures suggest that butyric acid is more effective at killing *Tylenchorhynchus* spp. when soil organic matter is present. The reasons for greater effectiveness are not immediately clear. Enhanced toxicity of butyric acid may be the result of compounds associated with organic matter that disrupt the integrity of the cuticle, facilitating acid diffusion. Alternatively, the presence of organic matter may result in microsites with localized low pH that would also facilitate acid diffusion.

A 7-day incubation in soil acidified with HCl to achieve a pH of 3.5 reduced *Tylenchorhynchus* spp. by 70% under aerobic conditions and 80% under anaerobic conditions relative to controls. However, incubation in soil-treated with 1 µmol butyric or propionic acid/g soil, resulting in similar pH values, eliminated nematodes completely. This indicates that a decrease in soil pH contributes to nematode death, particularly at lower pH values. Ruess and Funke (1992) reported a decline in bacterivorous nematodes following acidification of soil to pH values below 3.0 through addition of inorganic acids, whereas root and fungal-feeding nematodes actually increased over time. According to McSorley (1998) plant-parasitic nematodes appear to be unaffected by pH changes in the soil within the normal range. The values tested in the present study are below what is considered normal soil pH.

We expected fatty acids to be more effective under anaerobic conditions as mineralization of the organic acids by aerobic soil bacteria would be diminished in the absence of oxygen. Seven days following treatment, 0.1 and 1 µmol butyric acid and 1 µmol propionic acid/g soil had killed 100% of *Tylenchorhynchus* spp., whereas treatments exposed to lower concentrations of acid did not reduce numbers of nematodes significantly relative to the control, regardless of soil atmosphere. Carbon mineralization rates under aerobic conditions increased when butyric acid was added at low levels, which also were not nematicidal. These data suggest that soil microbes are able to metabolize the acids.

**Fig. 4.** CO$_2$ evolution following amendment of soil with butyric acid (0 and 10 µmol/g soil) under aerobic and anaerobic soil atmosphere. Values are means ± SD (n = 14). (*) Different from control (P ≤ 0.05).
that allow for their decomposition. In addition, the introduction of microbes from surrounding areas should facilitate fatty acid degradation, minimizing its persistence once it has had the intended effects. However, the low soil pH associated with nematicidal concentrations of butyric acid likely limits its use to pre-plant applications.

**Literature Cited**


