Effect of Carbon Amendment and Soil Moisture on *Tylenchorhynchus* spp. and *Hoplolaimus galeatus*¹

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Abstract: The effect of amending soil held at 3 different moisture levels with glucose, unsulfured molasses, or nutrient broth (0.3, 0.7, 3.2, 7.1 g carbon/100 g) on *Tylenchorhynchus claytoni* and *T. dubius* was investigated. When soil was held under saturated or flooded conditions in the absence of carbon amendments for 7 days, *Tylenchorhynchus* populations were 19% and 16%, respectively, of the controls. Carbon amendments at all levels tested precipitated a further decline in the nematode population to 1% or less of the unamended controls in 7 days. Two applications of molasses (7.4%, w/w) 3 days apart to nematode-infested soil held in Conetainers under mist for 7 days reduced *Tylenchorhynchus* spp. and *Hoplolaimus galeatus* densities to 7% and 3%, respectively, of the controls. Nematode densities in turfgrass field plots also declined following irrigation and repeated drenching with a molasses solution. Based on the observed decline in redox potential and pH in saturated soil, especially following carbon amendment, we propose that the activity of anaerobic fermentative bacteria was responsible for the reduction in nematode densities.

Key words: carbon, *Hoplolaimus galeatus*, moisture, molasses, nematode, pH, redox, *Tylenchorhynchus claytoni*, *Tylenchorhynchus dubius*.

Nematodes are recognized as serious parasites of both warm-season and cool-season turfgrasses (Nelson, 1995). Symptoms of nematode injury include yellowing, wilting, and dieback and usually appear during periods of heat-related stress (Mullin, 1993). The most effective strategy for managing nematodes is the development of a healthy stand of turfgrass that will tolerate high nematode populations. Stress factors to be avoided include soil compaction, nutrient stress, drought, and close mowing (Nelson, 1995). Options for chemical control are few. Concerns have arisen regarding the toxicity of synthetic nematicides to humans and wildlife, and their diminished effectiveness following repeated applications (Davis et al., 1993; Prakash et al., 1994; Rodriguez-Kabana, 1986; Rodriguez-Kabana and Morgan-Jones, 1987; Spiegel et al., 1989). Among the proposed mechanisms for nematode suppression by these management practices are toxic metabolites present in plant residues or toxins resulting from microbial decomposition of residues (Jalal and Read, 1983; Patrick et al., 1965; Sayre et al., 1965), and the production of high levels of ammonia (Rodriguez-Kabana, 1986). Alternatively, a slow decline in nematode populations has been observed in flooded rice paddy soils, and is thought to have resulted from the production of hydrogen sulfide (Rodriguez-Kabana, 1965). More rapid suppression of nematodes occurs in flooded soils following amendment with sucrose or cornmeal, presumably due to the fermentation products of anaerobic bacteria (Hollis and Rodriguez-Kabana, 1966; Johnston, 1959).

A laboratory study was conducted to determine the effect of amendment with carbon on plant-parasitic nematodes in soil held at 3 different moisture levels (60%, 100%, and 150% water-holding capacity [WHC]). Carbon amendments included glucose, molasses, and nutrient broth. The
Effect of adding molasses to saturated soil in the greenhouse and in the field also was investigated. We hypothesized that the addition of a readily degradable source of carbon to moist soil would result in the establishment of conditions conducive to the activity of anaerobic, fermentative bacteria that have been implicated in nematode suppression in flooded soil. To evaluate the dynamics of aerobic/anaerobic conditions in soil and their relationship to nematode control, we monitored the reduction-oxidation (redox) potential of the soil. Values of redox potential less than 200 mV indicate anaerobiosis (Tate, 1995). We measured changes in soil pH because the concentration of H⁺ has been implicated as an important variable controlling the toxicity of various microbially produced nematicidal compounds in soil (Banage and Visser, 1965).

Materials and Methods

Effect of soil moisture and carbon amendment on nematodes in microcosms: Soil (92% sand, 3% silt, 5% clay; pH 4.4; 3.8% OM), consisting of cores from golf course greens infested with Tylenchorhynchus claytoni (Kofoid and White) Chitwood and T. dubius (Bütschli) Filipjev, was sieved through a 6-mm-pore screen and mixed well to ensure even nematode distribution. Plastic, 140-ml specimen cups (Kendall Healthcare Products, Mansfield, MA) were filled with 100 g (dry weight) soil.

Three moisture levels were tested for their effect on nematodes with and without carbon amendments: 60%, 100%, and 150% water-holding capacity (26%, 43%, and 65% moisture, v/w, respectively). Carbon treatments consisted of glucose (Fisher Scientific, Fair Lawn, NJ), “unsulfured” molasses (Grandma’s Molasses, Mott’s USA, Stamford, CT), and nutrient broth (Difco Laboratories, Detroit, MI). Amendments were applied at rates of 0.3, 0.7, 3.2, and 7.1 g carbon/100 g soil, values which correspond with 0.80, 1.78, 8.01, 17.80 g glucose; 0.76, 1.69, 7.60, 16.90 g molasses; and 0.57, 1.26, 5.67, 12.60 g nutrient broth/100 g soil. Treatments were replicated 6 times. The carbon content of organic amendment solutions was determined with a Shimadzu TOC-5000 total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD). An appropriate volume of distilled, deionized water (DDW) was added to each carbon treatment to reach 60%, 100%, and 150% WHC. Treatments were added to soil and stirred well. Thereafter the soil was left undisturbed. Specimen cups were weighed immediately following amendment with carbon, and water was added every other day as needed to maintain the initial soil moisture level. Samples were incubated in the dark at 19 to 21 °C.

The number of mobile T. claytoni and T. dubius present in whole soil samples was determined 7 and 14 days following treatment. Nematodes were extracted with a modified Baermann tray apparatus consisting of a disposable aluminum pie plate fitted with screen (1-mm-pore) and lined with 1-ply tissue (Scotties, Scott Paper, Philadelphia, PA), resting on an aluminum cake pan that contained just enough DDW to wet the screen, and covered with a second cake pan to prevent drying. Twenty-four hours later, the water was decanted and passed through a 45-µm-pore sieve. Nematode suspensions were stored at 4.5 °C until counted. Data were subjected to a three-way ANOVA (SPSS, Chicago, IL). All pairwise multiple comparisons of means were performed with the Tukey test.

A duplicate set of treated soil samples (replicated 3 times) was prepared to allow for measurement of soil pH (1:1; Hendershot et al., 1993) 2, 5, 8, 11, and 14 days following treatment. Redox potential (Eh) was measured 1, 4, 7, 10, and 13 days following treatment for 3 samples/treatment. Eh readings were adjusted (+240 mV) for the use of a calomel reference electrode.

Molasses amendment of soil held under mist: Cores removed from several golf course greens infested with T. claytoni, T. dubius, and Hoplolaimus galeatus (Cobb) Thorne were sieved through a 6-mm-pore screen and combined with sieved fairway soil (55% sand, 37% silt, 7% clay; pH 5.7; 5.4% OM). Containers (4 cm × 20.5 cm, 152 ml; Stew
and Sons, Corvallis, OR) were lined with 6-cm-diam. circles of weed fabric (Magic Mat, Agri-Tex, Danbury, CT) and filled with 101 g (dry weight) soil.

Soil in each Conetainer was treated with 7.5 g (3.2 g C) molasses (59,688 kg molasses/ha) diluted with DDW to reach 100% WHC (61%, v/w). One-half of the treated Conetainers received a second dose of 7.5 g molasses 3 days later. Treatments and controls were replicated 6 times. Conetainers were held in a greenhouse under mist (activated every 5 minutes for 15 seconds from 0700 to 1800 hours) to maintain saturated conditions. Leachate from Conetainers was collected and examined for the presence of nematodes. Unsaturated, untreated controls (50% WHC) were maintained on a neighboring greenhouse bench. Temperature in the greenhouse ranged from 9 to 42 °C (mean 29 °C). Seven days after initial treatment, soil samples were placed in Baermann trays for nematode extraction. Nematode counts were subjected to a one-way ANOVA and means were separated with the Tukey test.

Effect of irrigation and amendment with molasses in field plots: A field trial was conducted at the University of Rhode Island in turf plots comprised of Poa annua and Agrostis palustris and infested with Tylenchorhynchus dubius and Heterodera galeata. The soil was Bridgehampton silt loam with pH 5.4 and 3.8% organic matter.

Molasses (1.5 kg; 630 g C), diluted in 3 liters of water, was applied to each of four 61-cm × 122-cm plots (19,730 kg/ha) on 29 and 30 September and 2, 4, 6, 8, and 10 October 1997 with watering cans. The four control plots received a drench of 4 liters of water. Plots were irrigated each evening with a spike impulse irrigation head from 1700 to 0700 hours (2 cm water/hour), and for 15 minutes immediately following treatment with molasses to prevent foliage burn. Four unirrigated control plots, located on either end of the irrigated plots, were protected from watering by a 1.4-m-high plastic sheet and a 20-cm buffer strip of turf. The mean ambient temperature from September 29 to October 31 was 11 °C (range of −7.2 to 28 °C). Mean daily soil temperature was initially 21 °C but declined to 6 °C by 31 October.

Redox potential (Eh) at a depth of 20 cm and pH were determined on all sampling dates. Nematode densities were evaluated on 25 September and 3, 10, 17, and 31 October. Five, 2.5-cm × 15-cm cylindrical cores were removed from each plot. Soil was sieved through a 6-mm-pore screen, and nematodes were extracted from the soil with centrifugal flotation (Zuckerman et al., 1990). Nematode counts were transformed with log_{10}(x + 1) to normalize distribution of data, and analyzed with a one-way ANOVA. Mean nematode counts from irrigated plots were compared to those from unirrigated plots with the Bonferroni test. Data sets with non-homogeneous variance were analyzed with a Kruskal-Wallis one-way ANOVA on ranks (SPSS, Chicago, IL).

Results

Effect of soil moisture and carbon amendment on nematodes in microcosms: Soil moisture and carbon amendment independently affected plant-parasitic nematode populations (P < 0.001), as did their interaction (P < 0.001). Across treatments, nematode densities in soil held at 60% WHC were significantly higher than those in soil held at 100% and 150% WHC (P < 0.05). There was no significant difference in nematode densities in soil held at 100% WHC compared to those in soil held at 150% WHC. Number of days after treatment (7 vs. 14) was not a significant factor.

Maintaining soil moisture at 100% WHC (saturation) was detrimental to nematode survival compared to soil held at 60% WHC (P < 0.01). Seven days in unamended, saturated soil reduced the average numbers of Tylenchorhynchus spp. by 81% compared to unsaturated soil (Fig. 1). Soil Eh declined steadily over the course of 14 days when soil moisture was held at 100% WHC (Fig. 2), becoming anaerobic (<200 mV) in 10 days. There was a gradual increase in soil pH (Fig. 2).

Amending soil with carbon had a negative impact on nematodes at all moisture levels.
tested. At 60% WHC, all treatments resulted in significantly fewer nematodes than the control in 7 days ($P < 0.01$) (Fig. 1). Overall, nutrient broth was the most detrimental to nematodes. All rates of nutrient broth consistently lowered soil redox potential and raised pH up to 9.2 (Fig. 3). By contrast, high rates of glucose (3.2, 7.1 g C) and molasses (7.1 g C) reduced soil pH from an initial 4.4 to $\leq 3.2$ (Fig. 3C–D).

Carbon additions to soil held at 100% WHC enhanced the decline in nematodes (Fig. 1) compared to unamended, saturated soil ($P < 0.01$). The average Tylenchorhynchus spp. population was 1% or less of the unamended controls (60% WHC) 7 days after adding carbon at all levels tested. A comparison of moisture levels within treatments revealed that low levels of glucose (0.3, 0.7 g C), molasses (0.3 g C), and nutrient broth (0.3 g C) were significantly more effective when soil was saturated or flooded ($P < 0.05$). The decline in soil redox potential was also enhanced through carbon amendment. Soil treated with either molasses or glucose (0.3 to 3.2 g C) exhibited a rapid decline in redox potential, reaching $-200$ mV by day 4 (Fig. 2A–C). Evidence of a shift toward oxic conditions was apparent 7 days following treatment with molasses at the two lowest rates at 100% WHC (Fig 2A-B). Soil treated with glucose did not recover from anaerobiosis as quickly. The $E_h$ in soil treated with nutrient broth (0.7 to 7.1g C) also declined rapidly (Fig. 2B–D), but the redox potential remained in the anaerobic region for the duration of the experiment. The pH of soil treated with nutrient broth and held at 100% WHC rose (up to 8.2), but not as high as in soil held at 60% WHC. Soil treated with low levels of molasses or glucose exhibited little change in pH, while higher rates resulted in a slow, steady decline in pH ($<4.0$) (Fig. 2C–D). All rates of molasses and glucose addition resulted in an initial increase in pH followed by a decline below initial levels (Fig. 2).

Molasses amendment of soil held under mist: Maintaining soil moisture at saturation for 7 days in the absence of carbon amendments reduced Tylenchorhynchus spp. and H. galeatus populations by 65% and 83%, respectively, compared to the 50% WHC controls ($P < 0.01$) (Fig. 4). Amending the soil with one application of 7.5 g molasses had no significant impact on nematodes, but two applications of molasses 3 days apart further reduced Tylenchorhynchus spp. and H. galeatus densities by 93% and 97%, respectively, in 1 week ($P < 0.01$). Less than 1% of the initial nematode population was found in the leachate.

Effect of irrigation and amendment with molasses in field plots: The T. dubius population in unirrigated control plots exhibited an initial decline, remaining constant throughout the rest of the trial period (Fig. 5). By contrast, irrigation resulted in a steady reduction in nematode numbers. Nematode decline was accelerated in plots receiving repeated applications of molasses; however, differences
Fig. 2. Effects of soil held at 100% WHC and amended with glucose, molasses, or nutrient broth on redox potential ($E_h$) and pH.
Fig. 3. Effects of soil held at 60% WHC and amended with glucose, molasses, or nutrient broth on redox potential (Eh) and pH.
in nematode densities between the treated plots and the unirrigated control plots were statistically significant on only one date, 15 days after initial treatment \((P = 0.03)\). As observed for *T. dubius*, irrigation combined with repeated applications of molasses caused the *H. galeatus* population to decline sharply by day 15 (Fig. 5), the only sampling date on which differences were significantly different \((P = 0.01)\).

Soil \(E_h\) declined as a result of irrigation, more so when molasses was applied, although not to anaerobic levels (Fig. 5). There was little change in pH. Repeated drenching of irrigated plots with a molasses solution appeared to have no detrimental effect on the turfgrass.

**DISCUSSION**

We have demonstrated suppression of *Tylenchorhynchus* spp. and *H. galeatus* populations in soil held under saturated as well as flooded conditions for 7 days, an effect which is expedited by the addition of organic amendments rich in carbon, such as glucose or molasses. Johnston (1959) demonstrated that the production of organic acids by anaerobic bacteria, rather than oxygen deprivation, was responsible for a decline in *T. martini* densities in flooded soil. Addition of cornmeal or sucrose to flooded soil increased the level of microbial activity, enhancing the nematicidal effect. Hollis and Rodrı́guez-Kámbana (1966) also reported a dramatic reduction in nematode populations in flooded rice soil 3 to 4 days following amendment with corn meal. Banage and Visser (1965) contended that the nematicidal effect of organic acids was related to the form of the acid present, which in turn is controlled by pH. When the pH of the soil solution is below the pKa of the acid, most of the molecules will be present in their undissociated form, which presumably passes through the nematode cuticle. Inside the pseudocoelom, the acid dissociates, releasing \(H^+\). This theory is supported by the absence of nematicidal activity of organic acids at neutral to alkaline pH (Dijan et al., 1994).

We observed a rapid decline in redox potential (below −200 mV) to levels conductive to anaerobic microbial activity, and a decline in pH to levels below the pKa for butyric and propionic acid (4.86 and 4.87, respectively) in soil amended with glucose or molasses and maintained under saturated or flooded conditions. These results suggest that the production of nematicidal levels of organic acids by anaerobic bacteria may be a plausible explanation for the nematode reduction observed in our study.

Why were nematodes adversely affected by high rates of glucose or molasses when soil was held at 60% WHC? There are two likely explanations: (i) the establishment of anaerobic microsites and subsequent microbial production of organic acids via fermentation, or (ii) osmotic effects. Formation of anaerobic zones within soil aggregates is controlled by the relative rates of microbial respiration and oxygen diffusion, which in turn are controlled by the amount of readily available carbon sources.
degradable organic carbon and moisture (Rowell, 1981; Smith, 1980). The decline in pH observed in soil samples treated with glucose and incubated at 60% WHC supports the notion that organic acids were produced during incubation. Takai and Kamura (1966) reported the presence of low levels of organic acids resulting from continuous addition of glucose (0.1%) to dry, oxygenated soil.

An alternative explanation for nematode reduction, especially since the effects of molasses and glucose were greatest at the highest treatment rates, is plasmolysis of the nematodes. Feder (1960) reported that the addition of 5% sucrose or dextrose (w/w) to soil resulted in up to 100% nematode kill in 24 hours. Since death was rapid following immersion in sugar solutions, he concluded it resulted from an osmotic effect. Blake (1961) reexamined the rates employed by Feder (1960) and concluded that the solute concentration necessary to induce plasmolysis is comparable to the permanent wilting point for plants. Therefore, even if the application of sugar was practical and economically feasible, it could be used only to treat fallow fields. The rates of glucose employed in our microcosm trial (Experiment 1) ranged from 0.8% to 18% (w/w), so plasmolysis of the nematodes in soil treated with the highest amendment rates (3.2 and 7.1 g C/100 g soil) may have occurred.

Nitrogen-rich nutrient broth was effective...
in suppressing nematodes, probably via a different mechanism. In this case, the production and accumulation of toxic levels of ammonia, which is also thought to plasmolyze nematodes (Rodríguez-Kábana, 1986), may have been involved. Rodríguez-Kábana and Morgan-Jones (1987) recommended soil amendments with a low C:N ratio (<20:1) for reduction in nematode populations. There is a pH-dependent, dynamic equilibrium between NH$_4^+$ and NH$_3$ (Du Plessis and Kroontje, 1964). Neutral to alkaline conditions, which were present following treatment with nutrient broth, favored a shift in the equilibrium to ammonia, lending support to the involvement of ammonia as a nematicide.

Although prolonged flooding has been shown to reduce nematode populations, this approach is not feasible in turfgrass or most cropping systems. We have demonstrated that it is possible to achieve the same nematicidal effect observed in flooded soil in a shorter period of time by adding carbon to saturated soil. The quantity of water we applied to field plots, however, was excessive and should be reduced to minimize leaching of carbon compounds, an event we observed in the Conetainers and we suspect occurred in the field plots. Low soil temperatures may also have played a role in preventing a decline in soil redox potential to anaerobic levels in field plots.

We suggest that amendment with readily degradable carbon sources, such as glucose or molasses, facilitates a rapid decline in redox potential, and the concomitant activity of anaerobic, fermentative bacteria results in the production of nematicidal substances. Based on the rapid decline and subsequent recovery of soil Eh in microcosms following treatment of saturated soil with molasses (0.3 g C/100 g), it appears that the anaerobic period required for the production and accumulation of toxic levels of nematicidal substances is relatively short (a week or less). Amendment of soil with a readily degraded carbon source to achieve nematode suppression appears to be a viable management strategy that warrants further investigation.

**Literature Cited**


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