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Effectiveness of Liquid Hog Manure and Acidification to Kill *Pratylenchus* spp. in Soil

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Abstract: The effectiveness of liquid hog manure (LHM) and Acidified LHM to kill *Pratylenchus* spp. in potato soils was examined using two micro-plot and one field experiment. Micro-plot experiments were conducted in 2004 and 2005 using two slightly acid soils (designated BPF and MS) harbouring *Pratylenchus* spp. Treatments applied to the two soils in 2004 were; Control, Sulfuric acid (equivalent to 1,800 liters ha^{-1}), LHM (equivalent to 58,500 liters ha^{-1}), and Acidified LHM (equivalent to 2,400 liters sulfuric acid plus 58,500 liters LHM ha^{-1}). Treatments were repeated in 2005. Sulfuric acid was used to reduce soil pH to 4.3 and 5.5 in 2004 and 2005, respectively. By harvest in 2004, only Acidified LHM reduced *Pratylenchus* spp. population in BPF and MS soils by 94% and 91%, respectively. By harvest in 2005, LHM and Acidified LHM reduced populations by 56% and 61%, respectively, for BPF, and by 60% and 93%, respectively, for MS soil. The field experiment was conducted in 2005 on a commercial potato field. Different combinations of LHM (equivalent to 58,500 liters ha^{-1}) and sulfuric acid were added to field plots. Post-application, LHM acidified to pH 6.2 and 5.5 reduced *Pratylenchus* spp. populations by 51% and 89%, respectively. However, Acid (pH 4.6) and LHM treatments reduced populations by 43% and 74%, respectively. By harvest, no treatments reduced populations compared to Control treatment. We conclude LHM and Acidified LHM killed *Pratylenchus* spp. in the slightly acid soils examined consistent with VFA toxicity being the mechanism; acidification seemed to enhance LHM to kill *Pratylenchus* spp. only when VFA concentration of LHM was low.

Key words: acidity, LHM, liquid hog manure, LSM, liquid swine manure, plant-parasitic nematodes, *Pratylenchus*, root-lesion nematode, toxicity, VFA, volatile fatty acids.

Pratylenchus spp., Filipjev (1936), have the widest host range among plant-parasitic nematodes and rank behind only root-knot and cyst nematodes in terms of their worldwide economic impact on crops (Sasser and Freckman, 1987; Duncan and Moens, 2006). Of the 68 *Pratylenchus* species, eight (*P. alleni*, *P. crenatus*, *P. neglectus*, *P. thornei*, *P. scribneri*, *P. coffeae*, *P. brachyurus*, and *P. penetrans*) are associated with poor growth of potato (*Solanum tuberosum*) in many production areas worldwide (Brodie et al., 1993; Ingham et al., 2005; Scurrah et al., 2005; Castillo and Vovlas, 2007). *Pratylenchus* spp. cause stunting and yellowing of potato plants and severe necrotic lesions in the roots and tubers (Brodie et al., 1993), resulting in yield loss as much as 25-73% (Olthof, 1986). In addition, three species, *P. crenatus*, *P. penetrans*, and *P. scribneri*, are capable of interacting synergistically with the wilt fungus, *Verticillium dahliae,* forming the disease complex known as Early Dying of Potatoes when population levels of the two pathogens are too low to cause disease alone (Riedel et al., 1985; Rowe et al., 1985; Rowe and Powelson, 2002). *Pratylenchus penetrans* is the most important *Pratylenchus* spp. that enhances the development of Early Dying of Potatoes (Castillo and Vovlas, 2007). Early Dying of Potatoes is a limiting factor in potato production in various areas in North America (Martin et al., 1982; Rowe et al., 1987).

Damage to potato caused by *Pratylenchus* spp. can be reduced by lowering pre-plant soil population levels to below the economic threshold population level (Viaene et al., 2006). Several management practices have been used to achieve this; however, some of these practices have limitations (Castillo and Volvas, 2007). Crop rotation can be used to minimize damage by *Pratylenchus* spp.; however, the wide host range of *Pratylenchus* spp. makes successful crop rotation difficult (Chen et al., 1995). Use of resistant crop cultivars is limited by the lack of resistant germplasm to incorporate into commercial cultivars (France and Brodie, 1995; Brodie, 1998). In addition, biological control agents have not proven to be effective in production systems (Hackenberg et al., 2000; Castillo and Volvas, 2007). Soil fumigation and nematicides are restricted to high-value crops due to the high cost (Olthof, 1987; Olthof, 1989; Rich et al., 2004; Duncan and Moens, 2006).

Manures have been added to agricultural lands for centuries as a source of essential plant nutrients and organic matter (Shulte, 1977). Some studies have shown anaerobically digested or stored organic wastes to kill plant-parasitic nematodes. Liquid hog manure (LHM), also called liquid swine manure (LSM), reduced populations of *Pratylenchus* spp. when incorporated into potato fields (Conn and Lazarovits, 1999), and reduced populations of plant-parasitic nematodes when surface-applied to grassland (Valocka et al., 2000). In a pot study, LHM added to soil inhibited egg production, hatch and survival of J2 stage juveniles of *Heterodera glycines* (Xiao et al., 2007 and 2008). Anaerobically digested cattle manure reduced damage severity to tomato as well as egg mass and population of *Meloidogyne incognita* in a pot study (Jothi et al., 2003). Also using a pot study, Min et al. (2007) found anaerobically

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digested cattle and swine manures to reduce populations of *P. penetrans* in an acid soil. Sequential sprinkler irrigation of liquid dairy manure to forage crops reduced populations of *Pratylenchus* spp. and *Paratrichodorus* spp. (Timper et al., 2004). In contrast, Forge et al. (2005) found populations of *P. penetrans* increased with annual application of LHM to forage in neutral pH soil.

Several studies have shown LHM to control plant pathogens (Tenuta et al., 2002; Conn et al., 2005). Application of LHM to potato fields reduced the incidence of verticillium wilt and potato scab, caused by *V. dahliae* and *Streptomyces scabies*, respectively (Conn and Lazarovits, 1999). LHM was shown to control plant pathogens through various mechanisms including the effects of ammonia, nitrous acid (Conn et al., 2005), and short-chain volatile fatty acids (VFA) (Tenuta et al., 2002; Conn et al., 2005). VFA in LHM are the products of bacterial anaerobic fermentation of carbohydrates and amino acids which takes place in the gastrointestinal tract of hogs and during LHM storage under anaerobic conditions (Zhu, 2000). In a survey of hog farms in southwestern Ontario, Canada, it was found that LHM from finishing hog operations had sufficient VFA to be used as an effective control product to *V. dahliae* while LHM from sow operations did not (Conn et al., 2007). This was due to the manure being more diluted with wash water in sow operations compared to finishing hog operations. Acidic conditions generating non-ionized forms of short-chain VFA (acetic, propionic, *n*-butyric, isobutyric, *n*-valeric, isovaleric and *n*-caproic acids) in LHM were shown to be responsible for the suppression of microsclerotia of *V. dahliae* (Conn and Lazarovits, 2000; Conn et al., 2005; Tenuta et al., 2002). More recently, using solution exposure studies, Mahran et al. (2008), concluded that VFA can account for the majority of the lethal effect of LHM to *P. penetrans* under acidic conditions. Also, liquid hog manure enriched in VFA was more effective in inhibiting egg production by *Heterodera glycines* than the manure unenriched in VFA (Xiao et al., 2007). In a laboratory experiment, short-chain fatty acids, butyric and propionic, reduced *Tylenchorhynchus* spp. population in unsaturated soil under both aerobic and anaerobic conditions (McElderry et al., 2005).

As part of our research program to evaluate LHM as a strategy to manage *Pratylenchus* spp., the ability of LHM and Acidified LHM to kill the nematodes in potato soils was examined using two micro-plot and one field experiment. LHM, either as is or acidified, was used to test whether the nematicidal effect of LHM occurs under field conditions and if acidification improves its effectiveness.

Materials and Methods

Micro-plot Experiment 2004: The experiment was conducted at the Southern Crop Protection and Food Research Centre (Agriculture and Agri-Food Canada, London, Ontario, Canada) in the spring of 2004. Forty-eight micro-plots made from pieces (25 cm i.d. × 25 cm height) of perforated drainage tile buried in soil vertically 20 cm were used. There were six replicate tiles per treatment arranged in a randomized block design. Soil from two commercial potato fields (soil designated as BPF and MS) in the province of Prince Edward Island, Canada, harboring *Pratylenchus* spp. was used. Soil was collected the second week of May, 2004 to a 15cm depth from each field and couriered to London, Ontario for use.

The soils had a mixed population of plant-parasitic nematodes including *Criconema* spp., *P. crenatus, P. penetrans*, and *Tylenchorhynchus* spp. In both soils, over 70% of plant-parasitic nematodes were *P. crenatus* and *P. penetrans*. We identified at the University of Manitoba Potato Pathology Laboratory, the two *Pratylenchus* spp. using PCR according to Al-Banna et al. (1997, 2004) and sequencing of the D3 expansion region of the 26S rDNA. Briefly, 20 individuals of mixed stages were handpicked and each placed into separate PCR reaction tubes containing 5 µl extraction buffer (0.1 mM Tris-HCl, 0.05 mM EDTA, 0.2 mM NaCl, 1% SDS, and 0.5 mg proteinase K ml^{-1}). The tubes were placed at −80°C overnight. The tubes were then heated in a water bath for 1 hr at 65°C, then heated at 95°C for 1 minute and at 99°C for 3 minutes in a PCR thermocycler (Techne Flexigene, Techne Inc., Burlington, NJ). The D3 expansion region of the 26S rDNA was amplified by PCR using the forward primer D3A (5′-GAC CCG TCT TGA AAC ACG GA-3′) and the reverse primer D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′). PCR products were separated by gel electrophoresis (1.7% agarose gel and ethidium bromide staining) and the resulting band excised from the gel, with the DNA extracted and purified using an extraction kit (QIAquick, Qiagen Inc., Mississauga, ON). The DNA products were then sequenced (Macrogen Corp., Rockville, MD). A Nucleotide-nucleotide BLAST search in the Genebank for the PCR product sequence of each of the 20 nematodes from each soil indicated excellent match of 8 to *P. penetrans* and 7 to *P. crenatus* (E= 2^{-92} to E= 3^{-93}), five did not yield a match to a sequence in the database.

Before setup of the experiment, each soil was thoroughly mixed and then slightly dried to bring soil moisture to about 12% (w H_2O w^{-1} dry soil), and 13 kg placed in polyethylene bags, one bag for each tile. The soil had been partially dried to allow addition of LHM and/or acid and mixing within bags. Drying was done by spreading soil onto a table for three hours at room temperature. Each soil was thoroughly mixed prior to drying and after drying to insure similar population of nematodes in 13 kg subsamples.

Soil amendments were added to each bag, mixed with the soil, and the soil placed in a tile. The pH was

6.6 and 6.1 for BPF and MS soil, respectively, organic matter content was 3.5 and 5.7% for BPF and MS soil, respectively, and the texture of the soils was sandy loam. The LHM used in this experiment was collected in spring 2004 from a below ground covered storage pit at a finishing hog barn in southwestern Ontario, Canada. The manure had 10.0% dry matter, 0.95% total N, 0.54% NH_4^+-N, 0.21% total P, and 0.47% total K concentration on an as-is basis. The total VFA concentration (ionized plus non-ionized forms) of the LHM was 149 mM (acetic, 81 mM; propionic, 29 mM; isobutyric, 6.4 mM; *n*-valeric, 8.1 mM; isovaleric, 7.9 mM; and *n*-caproic, 17 mM). The pH of each soil was reduced using sulfuric acid to a pH of 4.3 which was chosen to assure that there were more than sufficient concentration of non-ionized forms of the VFA to suppress *P. penetrans* based on previous 24 hour solution exposure studies (Mahran et al., 2008). The amounts of sulfuric acid needed to bring the pH of the soils and LHM down to pH 4.3 was determined by titrating samples of the soils and LHM with acid before setting up the experiment. Different combinations of sulfuric acid (98% concentrate) (Anchem Sales, London, ON) and LHM were added to soil on June 9, 2004 to provide the following treatments: Control (no sulfuric acid or LHM added), Sulfuric acid (12 ml per tile equivalent to 1,800 liters ha^{-1}), LHM alone (390-gm per tile or 3% of soil mass, equivalent to 58,500 liters ha^{-1}), and Acidified LHM (16.3 ml sulfuric acid equivalent to 2,400 liters ha^{-1} sulfuric acid plus 390-gm LHM). The Acidified LHM treatment required more sulfuric acid (4.3 ml per tile) than the Sulfuric acid alone treatment to prevent the LHM from raising soil pH above 4.3. The density of LHM was assumed to be 1-gm ml^{-1} with the addition rate used estimated being 58,500 L ha^{-1} assuming a soil bulk density of 1,300 kg m^{-3}, which is typical for sandy loam soil and incorporation depth of 15cm, also typical for surface applied followed by discing incorporation of LHM. The same soil bulk density and incorporation depth were assumed for estimates of equivalent application rates of acid ha^{-1}. Sulfuric acid was added to soil first followed by LHM to overcome foaming when the acid is mixed with LHM. One potato tuber, cv. Snowden, was planted in each tile one week after addition of amendments. The soil was fertilized one week after planting with an N/P/K granular fertilizer blend at a rate of 260/84/79 kg ha^{-1}, respectively. For the LHM treatments, the amount of N/P/K obtained from the manure was taken into account and the amount of inorganic fertilizer reduced accordingly so that the total N/P/K added was equal for all treatments. The crop was irrigated using a sprinkler system as needed during the growing season. In the fall, first week of October 2004, soil samples were collected from each tile for nematode analysis as described later in this paper, and tubers were weighed. All tubers within a tile were collected at harvest. Plants senesced and died naturally two weeks prior to soil collection and harvesting in this experiment and subsequent experiments presented here. Thus at time of soil collection plants roots were partially decomposed and likely endo-parasitic plant nematodes would have begun or completed migration to soil.

The soil pH and VFA concentration for each tile was determined immediately after treatment application (0 hrs) in June and pH again determined in October at harvest using 1:2 soil:water (w:w) extracts. The effect of treatments on total nematode numbers, *Pratylenchus* spp. numbers, and yield of tubers (> 3.17 cm width) at harvest are also reported.

Micro-plot Experiment 2005: The experimental set up was identical to 2004 except the initial soil pH after acid treatments was set to a higher pH being 5.5. The pH was set to 5.5 in 2005 for two reasons; (i) large amounts of sulfuric acid had been used in 2004 to lower the pH of the soil to 4.3, and (ii) in the acid only treatment in 2004, the soil pH did not return to control levels. Based on the pK_a values of individual VFA, the VFA concentration of the manure, and results of 24 hour solution exposure studies (Mahran et al., 2008), we expected there was still more than sufficient non-ionized VFA concentration in the Acidified LHM treatment to kill *Pratylenchus* spp. Different combinations of sulfuric acid (98% concentrate) and LHM were applied on June 9, 2005 to freshly collected BPF and MS soils to provide the following treatments: Control, Sulfuric acid (3.2 and 0.8 ml per tile equivalent to 500 and 112 liters ha^{-1} for BPF and MS, respectively), LHM alone (390-gm per tile), and Acidified LHM (4.5 and 2.1 ml sulfuric acid equivalent to 670 and 300 liters ha^{-1} for BPF and MS, respectively plus 390-gm of LHM per tile). The LHM was obtained in spring 2005 from the same storage pit as in 2004. The manure was more dilute than that used in 2004 having 2.2% dry matter, 0.29% total N, 0.22% NH_4^+-N, 0.03% total P, and 0.29% total K concentration on an as-is basis. Yet the total VFA concentration (ionized plus non-ionized forms) of the LHM used in 2005 micro-plot study was greater than that in 2004 being 261 mM (acetic, 162 mM; propionic, 54 mM; isobutyric, 15 mM; *n*-valeric, 6.2 mM; isovaleric, 16 mM; and *n*-caproic, 7.8 mM). In the fall, last week of September 2005, and after natural senescence of the plants for two weeks, soil samples were collected from each tile for nematode analysis as described in this paper, and tubers were weighed. The soil pH and VFA concentration for each tile was determined immediately after treatment application (0 hrs) in June and pH again determined in September at harvest using 1:2 soil:water (w:w) extracts. The effect of treatments on total nematode numbers, *Pratylenchus* spp. numbers, and tuber yield at harvest are also reported.

Field Experiment: During the last week of April 2005, an experiment was set up at a commercial potato field with loamy sand soil (pH 6.9, 3.2% organic matter) near Aylmer, Ontario, Canada. Different sulfuric acid

(50% concentrate) and LHM combinations were used to provide the following treatments: Control (no acid or LHM added), Sulfuric acid Treatment A (soil pH reduced to 5.8 using sulfuric acid equivalent to 3,400 liters ha^{-1}), Sulfuric acid Treatment B (soil pH reduced to 4.6 using sulfuric acid equivalent to 6,500 liters ha^{-1}), LHM (equivalent to 58,500 liters ha^{-1}), Acidified LHM treatment A (soil pH reduced to 6.2 using sulfuric acid equivalent to 4,000 liters ha^{-1} plus LHM equivalent to 58,500 liters ha^{-1}), and Acidified LHM treatment B (soil pH reduced to 5.5 using sulfuric acid equivalent to 4,800 liters ha^{-1} acid plus LHM equivalent to 58,500 liters ha^{-1}). The LHM was obtained in spring 2005 from the same storage pit as for the micro-plot experiments. The manure was more similar to that used in the 2005 micro-plot study having 1.6 % dry matter, 0.26% total N, 0.21% NH_4^+-N, 0.03% total P, and 0.26% total K concentration on an as-is basis. The VFA concentration (ionized plus non-ionized forms) in the LHM used in the field experiment was even greater than previously used in the micro-plot experiment being 367 mM (acetic, 233 mM; propionic, 70 mM; isobutyric, 20 mM; *n*-valeric, 9.4 mM; isovaleric, 25 mM; and *n*-caproic, 9.7 mM). Sulfuric acid (diluted 10-fold in water) and LHM were directly injected into the soil to a 15 cm depth using a liquid manure tanker (20,000 liter capacity) fitted with an injector/cultivator tool bar (38 cm spacing between injectors and depth of cultivation 15 cm). In the treatments receiving both sulfuric acid and LHM, the acid was applied first followed by LHM.

The experimental design was a randomized block design with four replicate plots (15 × 5.5 meters) per treatment. There was a 9 m border between each replicate block. The field was clean of weeds prior to setup of the experiment. From each treatment plot, ten soil subsamples were collected pre-application, post-application (Day 0) and at harvest using a soil core sampler (2.5 cm diameter) to 15 cm and then combined together for a sample occasion. The post-application collection of soil occurred between 3 and 7 days following application because of inability to complete sampling in one day and arrangement of crew to sample. Previous microcosm experiments showed maximum mortality of *P. penetrans* occurred by 3 days of adding Acidified LHM (Mahran et al., *submitted*) and thus the post-application sampling was expected to capture mortality resulting from treatment addition. Two weeks following treatment, six rows of potato cv. Snowden were planted in each plot (90 cm between rows and seed potato planted 30 cm apart within row) at a depth of 15 cm. The plots were hilled once after planting. No extra fertilizer was applied to the plots receiving LHM; however, the other plots received a typical amount of fertilizer (N/P/K at a rate of 224/84/79 kg ha^{-1}). The site was irrigated throughout the potato growing period as needed using a center-pivot system. In the fall, second week of October, 2005, the middle two rows of each plot were harvested and the tuber yield determined. Soil pH was determined immediately post-application (Day 0) and at harvest as previously described. The effect of the treatments on total nematode and *Pratylenchus* spp. numbers at pre-application, post-application (Days 3 to 7) and harvest are reported. In addition tuber yield (> 3.17 cm width) at harvest is reported.

Nematode Analysis: Soil samples were placed on ice immediately upon collection and sent by overnight courier to the Soil Ecology Laboratory at the University of Manitoba for nematode extraction and analysis. The samples were then stored at 5 °C and processed within 1 week of being received. A 100-gm fresh weight subsample of each soil was used for nematode extraction and analysis. Nematodes were extracted using Cobb's sieving and decanting (using USA Standard Test Sieve 100 then 400 mesh) followed by sugar flotation (using USA Standard Test Sieve 500 mesh) (Ingham, 1994). Nematodes were then placed in a gridded plastic Petri dish for nematode total number determination. The total number of nematodes in each dish was counted using a dissecting microscope at 80× magnification. Numbers of *Pratylenchus* spp. were determined by identifying 100 nematodes to the genus level using an inverted compound microscope at 400× magnification.

pH and Volatile Fatty Acid Analysis: The entire contents of replicate tiles for the micro-plot experiments were placed in polyethylene bags after harvest and the soil thoroughly mixed. The composite of core samples for a field plot were also mixed in a polyethylene bag. A subsample (8 g) of soil was then added to cold water (40 ml) in polyethylene bags (Seward Stomacher Blending Bags; VWR International, Edmonton, AB). The bags were heat sealed and the slurry was mechanically disrupted (30 s) using a Stomacher homogenizer (Seward Medical, Worthing, UK). The bags were placed on an orbital shaker (200 rpm) for 1 h at 4°C. The bags were removed from the cold, mechanically disrupted again, and allowed to sit at room temperature for 1 h. The pH of the standing solution was determined using a polymer body pH electrode (Cole-Parmer Canada Inc., Montreal, PQ).

The concentrations of ionized plus non-ionized forms of individual VFA in LHM and soil extracts were determined by ion exclusion chromatography using chemical suppression and conductivity detection (Dionex model 100, Dionex Corp., Sunnyvale CA). Following pH determination, a portion of the standing solution (1.5 ml) was transferred to microcentrifuge tubes and particulates in the solution were removed by centrifugation (10 min at 10,600 *g*). The analytical column used was an IonPac ICE-AS1 along with an AMMS ICE II chemical suppressor (Dionex Corp.). Extract solution contained in vials was introduced to the ion chromatograph using an autosampler equipped with a refrigerated chamber housing the vials (Waters 717 plus, Waters Associates, Milford, MA). The concentrations (in mM)

of non-ionized VFA were estimated using the concentration of ionized and non-ionized individual VFA, soil pH, soil moisture and the Henderson-Hasselbalch equation as previously described (Conn et al., 2005).

Statistical Analysis: For the micro-plot experiments, the values presented for total nematode population, *Pratylenchus* spp. population, tuber yield, pH, and VFA concentration are the means of six independent replicate tiles. The data were tested for normality and subjected to ANOVA. The ANOVA showed no block effects, but treatment effects were significant. Means of treatments were compared using the Student-Newman-Keuls method ($\alpha < 0.05$) using the GLM procedure of the SAS computer software package (SAS Institute Inc., Cary, NC). For the field study, the values presented for total nematode population, *Pratylenchus* spp. population, tuber yield, and pH are the means of four independent replicate plots. The data were tested for normality and subjected to ANOVA. The ANOVA revealed that there was no block effect. The means of treatments were compared as described for the micro-plot experiment.

Results and Discussion

This study examined the effectiveness of LHM to kill *Pratylenchus* spp. in soil. It further tested the hypothesis that acidification of LHM would improve efficacy by promoting the presence of lethal, non-ionized forms of VFA.

In the 2004 micro-plot experiment, Acidified LHM significantly reduced the *Pratylenchus* spp. population ($P < 0.05$) at harvest by 94% and 91% compared with the Control treatment in BPF and MS soils, respectively (Table 1). In addition, it reduced the total nematode population ($P < 0.05$) by 50% and 38% in BPF and MS soils, respectively (Table 1). In contrast, application of LHM without acidification affected neither *Pratylenchus* spp. nor total nematode populations in the two soils (Table 1). Sulfuric acid treatment alone had no effect on total nematode population but increased *Pratylenchus* spp. population ($P < 0.05$) in BPF soil (Table 1). The increase in *Pratylenchus* spp. population in soil receiving the Sulfuric acid treatment alone is possibly related to the optimum pH for *P. penetrans* reproduction being 5.2 to 6.4 (Morgan and MacLean, 1968; Willis, 1972). The soil pH by the fall in the Sulfuric acid treatment was 5.7 and 5.6 for BPF and MS soils, respectively, lying within the optimum soil pH range for *P. penetrans* reproduction compared to the Control treatments being at the upper end of the range.

By harvest in the 2005 micro-plot study, Acidified LHM reduced ($P < 0.05$) the *Pratylenchus* spp. population compared to Control treatments by 61% and 93% in BPF and MS soils, respectively (Table 2). Total nematode population increased ($P < 0.05$) with Acidified LHM in BPF soil only. LHM addition alone also decreased ($P < 0.05$) *Pratylenchus* spp. populations by 56% and 60% for BPF and MS soils, respectively, but did not affect total nematode populations. Sulfuric acid alone treatment increased the *Pratylenchus* spp. population ($P < 0.05$) by 28% for the BPF soil but decreased ($P < 0.05$) total nematode population by 24%. The effect of LHM alone on *Pratylenchus* spp. population was not different ($P > 0.05$) from that of Acidified LHM in BPF soil; however, Acidified LHM was more effective in reducing ($P < 0.05$) *Pratylenchus* spp. population in MS soil than LHM alone.

The population of *Pratylenchus* spp. was not different ($P > 0.05$) between treatments prior to the start of the field experiment (Table 3). Within a week of application to soil in the field study, Sulfuric acid B (soil pH reduced to 4.6), LHM, and the two Acidified LHM treatments reduced ($P < 0.05$) *Pratylenchus* spp. populations to below that of the Control treatment (Table 3). The Acidified LHM B treatment reduced *Pratylenchus* spp. population by 89% and LHM, Acidified LHM A, and Sulfuric acid B treatments were reduced compared to Control populations by 74%, 51%, and 43%, respectively. However, by harvest, the population of *Pratylenchus* spp. between treatments was not different ($P > 0.05$). Similarly, total nematode populations were different

Table 1. Combinations of sulfuric acid and liquid hog manure (LHM) applied to BPF and MS soils in the 2004 micro-plot experiment. Shown are the mean total number of nematodes, root-lesion nematodes (*Pratylenchus* spp.) at harvest, and soil pH immediately after post-application (Day 0) and harvest, and also yield of tubers (> 3.17 cm width) at harvest.

Soil	Treatment[1]	Total nematodes (# kg^{-1} fresh soil)	*Pratylenchus* spp. (# kg^{-1} fresh soil)	Soil pH		Yield (g plot^{-1})
				Day 0	Harvest	
BPF	Control	15,500 a[2]	2,660 b	6.6 a	6.3 a	319 b
	Sulfuric acid	13,300 ab	4,160 a	4.3 b	5.7 b	355 b
	LHM	14,000 ab	1,600 b	6.5 a	6.6 a	419 b
	Acidified LHM	7,500 b	154 c	4.5 b	5.4 b	551 a
MS	Control	16,800 a	3,660 a	6.1 a	6.2 a	301 b
	Sulfuric acid	16,100 a	4,050 a	4.3 b	5.6 b	303 b
	LHM	15,700 ab	2,480 a	6.1 a	6.2 a	433 b
	Acidified LHM	10,300 b	320 b	4.4 b	5.1 c	522 a

[1]See text for details of treatments and their application rates for the 2004 micro-plot experiment.
[2]Values shown are the mean of six replicates. Values in a column for each soil followed by different letters are significantly different from one another ($P < 0.05$) as determined by the Student-Newman-Keuls test.

TABLE 2. Combinations of sulfuric acid and liquid hog manure (LHM) applied to BPF and MS soils in the 2005 micro-plot experiment. Shown are the mean total number of nematodes, root-lesion nematodes (*Pratylenchus* spp.) at harvest, and soil pH post-application and harvest, and also yield of tubers (> 3.17 cm width) harvest.

Soil	Treatment[1]	Total nematodes (# kg^{-1} fresh soil)	*Pratylenchus* spp. (# kg^{-1} fresh soil)	Soil pH		Yield (g $plot^{-1}$)
				Day 0	Harvest	
BPF	Control	7,620 b[2]	1,450 a	6.7 b	6.7 b	509 b
	Sulfuric acid	5,780 c	1,160 a	6.2 c	6.2 c	576 ab
	LHM	9,110 ab	640 b	7.3 a	7.4 a	760 a
	Acidified LHM	9,850 a	560 b	6.1 c	6.6 b	776 a
MS	Control	6,270 ab	800 b	6.0 a	5.9	499
	Sulfuric acid	5,750 b	1,020 a	5.7 b	6.0	666
	LHM	6,620 a	320 c	6.2 a	6.3	732
	Acidified LHM	6,860 a	60 d	5.6 b	5.9	772

[1]See text for details of treatments and their application rates for the 2005 micro-plot experiment.
[2]Values shown are the mean of six replicates. Values in a column for each soil followed by different letters are significantly different from one another ($P < 0.05$) as determined by the Student-Newman-Keuls test.

($P < 0.05$) between treatments for only the post-application sampling occasion (Table 3). At this time, Acidified LHM increased ($P < 0.05$) total nematode population by 314% compared to the Control treatment.

The yield of tubers in the 2004 micro-plot study was greater ($P < 0.05$) by 73% for each soil with Acidified LHM treatment compared to the Control treatment (Table 1). The yield increase was consistent with a decline in *Pratylenchus* spp. population with Acidified LHM indicating that soil levels of this pest or other pathogen/pest not studied here, could have been yield limiting. Tuber yield was greater in the 2005 micro-plot study ($P < 0.05$) 52% and 49% with LHM and Acidified LHM treatment to BPF soil compared to the Control treatment (Table 2). For MS soil, tuber yield was not statistically different ($P > 0.05$) between treatments though a numerical increase of 55%, 47%, and 33% was observed for the Acidified LHM, LHM, and Sulfuric acid treatments, respectively. In the field study, there was no difference ($P > 0.05$) in tuber yield of treatments (Table 3).

That LHM alone was effective in reducing *Pratylenchus* spp. population in soil in the 2005 micro-plot and field experiment indicated the manure can be active in killing the pest. Acid alone treatment did not lower populations of the pest except for an addition rate bringing soil pH very low, to 4.6 (Sulfuric acid B treatment). This indicates lowering soil pH, unless drastic, does not reduce populations of *Pratylenchus* spp. However, acid supplementation to reduce soil pH below 6, increased the effectiveness of LHM to kill *Pratylenchus* spp. as evident in population reduction in the 2004 micro-plot experiment and further lower population for Acidified LHM than LHM alone in the 2005 micro-plot experiment in MS soil. This indicates an interaction of acidification in somewhat improving the effectiveness of LHM to kill the pest.

Few studies have examined the effectiveness of LHM to kill plant-parasitic nematodes. Conn and Lazarovits (1999) showed LHM to reduce populations of *Pratylenchus* spp. in slightly acidic field soil. Xiao et al. (2007) showed VFA enriched LHM to be more effective in inhibiting egg production by *Heterodera glycines* than the manure un-enriched in VFA. They further demonstrated LHM inhibited *H. glycines* egg hatch and killed J2 stage of the pest in laboratory and greenhouse experiments (Xiao et al., 2008). LHM has been shown to control plant pathogens through various mechanisms including ammonia at soil pH > 8, nitrous acid (HNO_2) and non-ionized forms of VFA in soil of pH < 6 (Tenuta et al., 2002; Conn et al., 2005). The pH of soil used in

TABLE 3. Combinations of sulfuric acid and liquid hog manure (LHM) applied to a field experiment at a commercial potato field near Aylmer, Ontario, Canada. Shown are; the mean total number of nematodes and root-lesion nematodes (*Pratylenchus* spp.) pre-application, post-application (3 to 7 days), and harvest; soil pH immediately post-application (Day 0) and harvest; and also the yield of tubers (> 3.17 cm width).

Treatment[1]	Total nematodes (# kg^{-1} fresh soil)			*Pratylenchus* spp. (# kg^{-1} fresh soil)			Soil pH		Yield (kg 30 m^{-1})
	Pre-application	Post-application	Harvest	Pre-application	Post-application	Harvest	Day 0	Harvest	
Control	7,970	5,440 b[2]	5,200	1,750	1,220 a	350	6.9 a	5.7 ab	60
Sulfuric acid A	6,640	6,000 b	6,600	1,310	1,050 a	540	5.9 b	5.6 ab	60
Sulfuric acid B	8,120	9,430 b	7,050	1,120	690 b	70	4.6 d	4.9 b	44
LHM	6,640	3,920 b	7,160	1,040	320 bc	70	7.2 a	6.2 a	72
Acidified LHM A H LHM A	8,240	4,420 b	3,310	1,420	600 b	50	6.2 b	5.6 ab	53
Acidified LHM B	5,400	24,000 a	6,620	1,340	140 c	210	5.5 c	5.3 ab	59

[1]See text for details of treatments and their application rates for the field experiment.
[2]Values shown are the mean of four replicates. Values in a column followed by different letters are significantly different from one another ($P < 0.05$) as determined by the Student-Newman-Keuls test.

these experiments was less than 8, thus a mechanism of ammonia toxicity was unlikely.

The findings of this study are consistent with VFA being a mechanism for killing *Pratylenchus* spp. in soil. Acidification of LHM resulted in greater concentration of non-ionized VFA in soil (Table 4) and increased efficacy of the manure to kill the pest for both soils in the 2004 micro-plot experiment and for MS soil in the 2005 micro-plot experiment. Further, LHM used in the 2005 micro-plot and field experiment had 75% and 146% greater total VFA concentration than used in the 2004 micro-plot experiment. The LHM alone treatment in the 2005 micro-plot and the field study were effective in killing *Pratylenchus* spp. compared to Control treatments.

Non-ionized VFA were present in LHM and Acidified LHM soil treatments post-application in the micro-plot studies (Table 4). In 2005, the recovery of non-ionized VFA was lower in BPF than MS soil, and lower than for either soil in the 2004 microplot study. The *Pratylenchus* spp. population declined at the post-application sampling in BPF soil for the 2005 micro-plot study (Table 2) despite low concentration of non-ionized total VFA (0.1 mM). The low concentration of non-ionized VFA post-application was partly attributable to lower recovery of acetic acid plus acetate for the LHM alone treatment in BPF soil compared to Acidified LHM (5.5 compared to 40 mM, respectively) and compared to LHM (26 mM) and Acidified LHM (36 mM) in MS soil for the 2004 micro-plot study. There seems to have been either degradation of acetic acid plus acetate (Sorensen, 1998) or sorption to soil in BPF soil. The later has been demonstrated in marine sediments (Shiba et al., 2001) and thus likely possible in soil.

Consistent with a decline in acetic acid plus acetate at post-application was a rise in soil pH from 6.7 to 7.4 in BPF soil receiving LHM alone (Table 2). pH of LHM is controlled by relative amounts of total VFA: (NH_3+NH_4^+) (Paul and Beauchamp, 1989) where a decrease in total VFA results in a pH increase. Sorensen (1998) found a pH increase of 1 to 2 units for coarse-textured soil receiving VFAs. Thus unlike solution exposure studies in which VFA concentrations can be kept constant, in the 2005 micro-plot study, rapid loss of acetic acid plus acetate and subsequent rise in pH resulted in low concentration of acetic acid. Thus it is possible determined non-ionized VFA concentration post-application was lower than for that in soil at time of sampling, thus, underestimating exposure concentration of non-ionized VFA to *Pratylenchus* spp.

The results indicate that variation in the concentration of VFA in LHM from even a single source has implications if application without acidification will be effective in killing *Pratylenchus* spp. Variation in VFA concentration from different operations was documented in southwestern Ontario, Canada, where it was found that LHM from finishing hog operations had sufficient VFA to kill microsclerotia of *V. dahliae* while LHM from sow operations did not (Conn et al., 2007). This was attributed to the use of greater amounts of wash water in sow compared to finishing hog operations. The results here indicate the need for determining variation in VFA concentration in stored LHM within operations if to be used as a pest management strategy.

We cannot rule out that mechanisms other than VFA contributed to the effectiveness of LHM to kill *Pratylenchus* spp. in this present study. The soils used were slightly acidic and thus could have allowed nitrous acid accumulation (Tenuta and Lazarovits, 2002; Tenuta and Lazarovits, 2004) as ammoniac nitrogen nitrified. Recently we demonstrated that Acidified LHM was slightly more lethal to *P. penetrans* than a mixture of its acidified VFA (Mahran et al., 2008). LHM can contain VFA > C6, indoles, phenols, volatile amines, and sulfur-containing compounds (Zhu, 2000) that may be lethal to plant-parasitic nematodes, though, untested. The application rates of LHM used in these experiments are typical of that applied to cropped soil. Determining if these other compounds promote the effectiveness of

TABLE 4. Combinations of sulfuric acid and liquid hog manure (LHM) applied to BPF and MS soils in micro-plot experiments in 2004 and 2005. Shown are the Total Volatile Fatty Acid (VFA; ionized plus non ionized forms) in soil, Total VFA concentration in soil water, and non-ionized VFA in soil water immediately post-application (Day 0).

		Total VFA (mg kg^{-1} dry soil)		Total VFA (mM in soil water)		Non-ionized VFA (mM in soil water)[2]	
Soil	Treatment[1]	2004	2005	2004	2005	2004	2005
BPF	Control	0 a[3]	0 a	0 a	0 a	0 a	0 a
	Sulfuric acid	0 a	0 a	0 a	0 a	0 a	0 a
	LHM	59 b	50 ab	25 b	19 b	1 b	0.1 b
	Acidified LHM	84 b	124 b	38 c	61 c	24 c	3 c
MS	Control	0 a	0 a	0 a	0 a	0 a	0 a
	Sulfuric acid	0 a	0 a	0 a	0 a	0 a	0 a
	LHM	54 b	86 b	22 b	41 b	1 b	2 b
	Acidified LHM	93 c	119 b	47 c	56 b	33 c	8 c

[1]See text for details of treatments and their application rates for the 2004 and 2005 micro-plot experiments.
[2]Concentration of total non-ionized VFA in soil taking into account soil moisture and pH.
[3]Values shown are the mean of six replicates. Values in a column for each soil followed by different letters are significantly different from one another ($P < 0.05$) as determined by the Student-Newman-Keuls test.

LHM as-is to kill nematode pests and pathogens is interesting because foregoing the addition of acid to LHM would be more practical than acidification.

Although acidification somewhat improved the efficacy of LHM to kill *Pratylenchus* spp., its practical use by farmers is perhaps limited. Addition rates of sulfuric acid will need to be monitored carefully to prevent excessive acidification of soil. For example, lowering of pH of BPF and MS soils in the 2004 micro-plot experiment resulted in pH remaining lower than that of the Control treatment by harvest (Table 1). Such acidic soil pH may lead to increasing the availability of some elements (e.g. Fe and Mn) to toxic levels to the crops. At the same time, acidity may cause reduction in the availability of some nutrients (e.g., Ca, Mg, and P) (Martini and Mutters, 1985a; Martini and Mutters, 1985b).

In the 2004 micro-plot experiment, the acidification treatments decreased ($P < 0.05$) soil pH at post-application (Day 0) and by harvest sampling occasions. In the 2005 micro-plot experiment, acidification treatments did result in lowering ($P < 0.05$) soil pH immediately post-application (Day 0) (Table 2). However by harvest, soil pH was only lower ($P < 0.05$) for the Sulfuric acid treatment in BPF soil whereas it was higher with LHM alone treatment. In the field experiment, acidification treatments did result in lowering soil pH with the order of effectiveness of treatments being Sulfuric acid B, Acidified LHM B, Sulfuric acid A, and Acidified LHM A (Table 3). By harvest, soil pH was lower for the Control treatment than at post-application (Day 0) by almost one pH unit. A decrease in soil pH following nitrogen source addition for soil having low pH buffering ability is common as nitrogen addition as fertilizer or organic sources results in pH reduction through nitrification of added nitrogen (Bolan and Hedley, 2003; Chien et al., 2008) and soil pH returning to former levels in subsequent spring presumably following leaching over winter of acidity. At harvest, only the Sulfuric acid B treatment had lower ($P < 0.05$) soil pH than that of the Control.

In the field experiment we found addition of acid directly to LHM not possible because it resulted in foaming of the mixture, likely as a result of generation of non-ionized VFA gases. Instead, we applied concentrated sulfuric acid diluted 10 times in water to soil separate from LHM. Dilution was done to have enough volume to deliver the solution reliably to soil, enough volume of solution to mix within the top 15 cm of soil and to reduce the corrosiveness of the solution to the tank and application system. Clearly, a means of preventing foaming of Acidified LHM is required to increase the practicality of the mixture addition to soil.

The nematode community in soil receiving Acidified LHM treatments at harvest was by observation dominated by bacterial scavengers in the Cephalobidae and Rhabditidae and fungal-feeders in the Aphelenchidae and Aphelenchoididae. These nematodes are adapted to grow rapidly in response to growth of bacterial and fungal decomposers in soil (Bongers and Ferris, 1999). Their seeming dominance suggests that either Acidified LHM may have been toxic to the soil food web with nematodes in those families subsequently flourishing in response to microbial decomposers proliferating on dead microbial biomass or microbial biomass proliferating on readily available C in LHM. The impact of acidification of LHM on soil food webs needs to be determined to understand its effect on soil health.

LHM and Acidified LHM seem to be economically competitive to currently available fumigants used for *Pratylenchus* spp. control. Farmers can often obtain LHM at no cost from neighboring hog rearing operations requiring disposing of the material. Where disposal of LHM is not a concern, farmers receiving LHM often purchase it based on its value of plant nutrients. Estimated nutrient value of 58,500 L of LHM averaged for the three LHM products used here and based on average North American fertilizer market value from January 2007 to January 2008 is $412 USD (N=$0.88, P=$1.06 and K=$0.54 USD kg^{-1}; Oehmke et al. 2008). At the application rate, 58,500 liters ha^{-1}, and application cost of $0.25 CDND L^{-1} (Saskatchewan Agriculture and Food, 2006) being about $0.20 USD L^{-1}, the nutrient equivalent value and cost of LHM application is estimated at $533 USD ha^{-1}.

For soil acidification of LHM to be used by farmers to kill *Pratylenchus* spp., it must be more effective than LHM added alone. Clearly more studies are required to determine the benefit of acidification of LHM. In this study, there was improvement in the efficacy of LHM when acidified, being apparent in the 2004 micro-plot study using an LHM of lower VFA concentration than used in the 2005 micro-plot and the field study. If following further scrutiny, acidification of LHM holds up to improve the effectiveness of LHM in killing nematode pests, a low-cost source of acid would be required. Such a source is currently available as a waste product from metal cleaning and metal pickling industries (Kobe and Fredrickson, 1956). Currently, these waste solutions are used as a source of ferrous sulfate. The estimated cost of sulfuric acid solution based on cost of ferrous sulfate solution (source: http://www.theinnovationgroup.com) and rate of application of 1,800 L ha^{-1} is $420 USD ha^{-1}. Accordingly, the total cost of Acidified LHM application (LHM to 58,500 L ha^{-1}) would be $953 USD ha^{-1} if the LHM was only available for a cost. In comparison, the cost of a fumigant (e.g. Vapam (metam sodium)) application to control *Pratylenchus* spp. is approximately $1,400 USD ha^{-1} (based on 2008 prices and rate of application of 700 L ha^{-1}) (Dr. Robert Wick, personal communication). Accordingly, LHM application alone or acidified is a competitive option for *Pratylenchus* spp. control compared to fumigants. However, further testing of the effectiveness of LHM and necessity of acidification is needed.

In conclusion, micro-plot and field experiments showed that LHM is effective in reducing *Pratylenchus* spp. populations in at least the slightly acid soils examined here. The effectiveness of LHM was somewhat improved with acidification where the LHM was low in VFA concentration. The results of this study are consistent with VFA as the constituents responsible for toxicity of LHM to *Pratylenchus* spp. Further, application of LHM alone or acidified for *Pratylenchus* spp. control is potentially economically competitive to fumigants, however further studies are required to compare the effectiveness of LHM and acidification of LHM to fumigants.

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