Impacts of Sustained Use of Dairy Manure Slurry and Fertilizers on Populations of *Pratylenchus penetrans* under Tall Fescue

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Abstract: Various manures and composts have been reported to reduce population densities of plant-parasitic nematodes. Dairy manure slurry is often used as a primary source of nitrogen for forage crops. This study was conducted to determine the effects of dairy manure on population densities of *Pratylenchus penetrans* parasitizing tall fescue. Beginning in 1994, dairy manure and inorganic fertilizer were applied after each harvest (2 to 4 times/year) at rates of 50 and 100 kg NH$_3$N/ha; control plots were not treated. Nematode populations in soil and roots were determined at 19 sample dates during the fourth (1997), fifth (1998), and sixth (1999) years of manure and fertilizer applications. The sustained use of dairy manure and fertilizer increased population densities of *P. penetrans*. Our results contrast with many previous studies demonstrating that application of manures decreases population densities of plant-parasitic nematodes. Frequent applications of moderate amounts of manure to a perennial grass crop may have prevented the development of nematode-toxic levels of ammonia or other toxic substances such as nitrous acid or volatile fatty acids. Two years with no additional manure applications were required for *P. penetrans* population densities to return to levels similar to fertilized or untreated soil.

Key words: forage production, host-parasite interaction, manure, nematode ecology, nematode suppression.

Manures are important sources of nutrients for sustainable crop production. However, in regions with especially high livestock densities, quantities of manure applied to local crops can be substantial, leading to concerns about losses of nitrogen (N) to the environment and long-term effects on various aspects of soil quality. In south coastal British Columbia, grass swards are often fertilized with dairy manure slurry at cumulative rates of up to 500 kg NH$_3$N/ha/yr (1 t (m) total N/ha/yr), applied 3 to 5 times/season. The benefits of dairy manure N for forage growth and nutrient uptake (Bittman et al., 1999a), and losses of manure N via microbial immobilization (Bittman et al., 2005), leaching, and nitrous oxide emissions (Paul and Zebarth, 1997) have been studied extensively. Relatively little research has considered impacts of long-term use of dairy manure on non-nutrient aspects of the quality of soil for crop growth, such as the prevalence or population dynamics of soilborne pathogens.

Root-lesion nematodes, *Pratylenchus* spp., are common parasites of forage crops grown in temperate regions (Cook and Yeates, 1993; Kimpinski and Thompson, 1990). *Pratylenchus* spp. can limit production of forage legumes (Cook and Yeates, 1993; Kimpinski and Thompson, 1990; Minton, 1965; Thies et al., 1995) as well as forage grasses such as tall fescue (Cook and Yeates, 1993; Hoveland et al., 1975; Minton, 1965; Thies et al., 1995). For example, root biomass of tall fescue and perennial ryegrass were collectively 1.4 times greater in nematicide (carbofuran)-treated microplots than in nontreated microplots (Thies et al., 1995).

Populations of plant-parasitic nematodes have been suppressed by application of farmyard manure (Stirling et al., 1995), swine manure (Bulluck et al., 2002; Conn and Lazaro, 1997), poultry manure (Conn and Lazaro, 1997), and municipal biosolids (Castagnone-Sereno and Kermarrec, 1991), and composts made of cattle manure (Oka and Yermiyahu, 2002) and poultry manure (Abawi and Widmer, 2000). Other studies have demonstrated increased or unchanged populations of plant-parasitic nematodes in soil amended with beef manure (Kimpinski et al., 2003) and municipal composts (McSorley and Gallaher, 1995, 1996, 1997). Fertilization with inorganic sources of N also has been observed to increase populations of plant-parasitic nematodes. For example, under field conditions, Sarathchandra et al. (2001) reported that populations of *Pratylenchus* and *Paratylenchus* were increased in N-fertilized clover-ryegrass pastures. Similarly, Todd (1996) found that populations of *Paratylenchinae*, but not *Helicotylenchus*, increased in fertilized tallgrass prairie, and Dmowska and Ilieva (1995) reported that *Paratylenchus* spp. were more abundant in plots of barley fertilized with N over 22 years than in nonfertilized plots.

Understanding responses of forage crops to sustained use of manures and fertilizers would be improved by knowledge of the comparative impacts of these nutrient management options on *Pratylenchus* populations. Given the multiplicity of possible responses of *Pratylenchus* to N-rich amendments, it is not clear whether application of dairy manure slurry at approximately 100 kg NH$_3$N/ha at 3 to 5 times/year would result in increased or decreased *Pratylenchus* population densities. In previously published research we compared the effects of sustained use of fertilizer and dairy manure on dynamics of the microbial biomass (Bittman et al., 2005) and on populations of protozoa and non-phytoparasitic nematodes (Forge et al., 2005). In this paper we report on the effects of sus-
tained use of fertilizer and dairy manure on population dynamics of *Pratylenchus penetrans*. To better understand potential impacts on other crops that may be rotated with the grass sward, we also assessed the persistence of manure- or fertilizer-induced changes in *P. penetrans* population densities.

**Materials and Methods**

*Field plots:* Field plots sampled in this study were part of a multi-faceted study of the long-term effects of applying dairy manure slurry to grassland at different rates and frequencies over multiple years. The study was established on the grounds of the Pacific Agri-Food Research Centre in Agassiz, British Columbia (49°20' latitude, 121°46' longitude, 15 m elevation), which has a coastal, wet temperate climate. Average January and August temperatures are 2.1 and 18.1 °C, respectively, and average annual precipitation is 1,730 mm, with 70% of precipitation falling as rain between October and March. The primary grass species in the sward was tall fescue (*Festuca arundinacea* cv. Festorina) and the soil was a Monroe series, medium-textured, eluviated eutric brunisol (USDA, Albic horizon Eutrochrept) with about 6% organic matter, moderate to good drainage, and derived from medium-textured stone-free Fraser River deposit (Luttmerding and Sprout, 1967). Beginning in 1994, 3-m × 100-m strip plots were treated with manure or fertilizer 2 to 4 times/year. In all, there were 10 treatment combinations of application rate and frequency, randomized in four blocks. Only five of the 10 multi-year treatment combinations were considered in this study: i) manure at 100 kg NH₄-N/ha at each application, ii) manure at 50 kg NH₄-N/ha each application, iii) ammonium nitrate at 100 kg N/ha each application, iv) ammonium nitrate at 50 kg N/ha each application, and v) nontreated controls. The treatments were applied 2 to 4 times/year, in the spring and after each harvest except the last (Fig. 1). The fertilizer plots received P and K according to soil tests performed each spring. Approximately 50% of total manure N is NH₄-N, so the low N manure treatment received similar total N as the high N fertilizer treatment. In 1998, the fifth year of application, subplots were marked off from the end of each main strip plot, were nontreated, and were sampled separately to determine the persistence of manure- and fertilizer-induced changes in soil microfauna (residual fertility). Only the previously fertilized and previously manured plots were sampled in the residual fertility sub-experiment. The main plots contin-

![Graph showing population dynamics of *Pratylenchus penetrans* in soil treated with high rates of manure and fertilizer. Fertilizer and manure were both applied at 100 kg NH₄-N/ha each application. Arrows on the X-axis mark manure application dates. Vertical bars attached to manure data points are LSD values (*P* = 0.05) for comparing individual sample means within specific dates (*n* = 4).]({})
used to receive the treatments allocated to them over the previous 4 years (continuing treatments).

**Sampling and analyses:** Samples were taken 1 and 3 weeks after one of the manure applications in 1997, two manure applications in 1998, and all four manure applications in 1999. The other five sample dates (three in 1997 and two in 1998) were not linked with particular manure application dates. At each sample date, 25 2.5-cm diam. × 30-cm-deep cores were taken from each plot and combined to form a single composite sample representing each plot. The samples were refrigerated and processed within 7 days of sampling. Each soil sample was sieved (5 mm) to remove stones and root fragments. Rather than extracting *Pratylenchus* from roots and soil separately, root fragments collected on the sieve during the initial sieving were chopped into < 1-cm fragments and mixed back into the soil prior to subsampling and extraction. Baermann pans (16-cm diam.) were used to extract nematodes from 50-g subsamples of the soil with root fragments (Ingham, 1994). The nematodes were heat-killed and preserved in 4% formalin. One-third of each nematode suspension was observed on a Sedgewick-Rafter slide (FGR Steinmetz, Burnaby, BC) at 200X magnification and *Pratylenchus* counted. Nematode counts were adjusted to 100 g dry soil.

*Pratylenchus penetrans* was identified as the dominant species at the site on the basis of the presence of males in the population, round spermathecae, and three lip annules. *Pratylenchus* nematodes chosen for identification were individual gravid females that appeared to have spermathecae under the dissecting microscope, so our observations confirmed the presence of *P. penetrans* but we could not rule out the presence of other *Pratylenchus* spp. in the population.

**Data analyses:** The population data for each year were analyzed using a blocked split-plot-in-time analysis of variance model. Treatments were considered as main-plots and the multiple sample dates within each year were considered as sub-plots. The treatment × block sums of squares was used as the error term for testing the effect of treatment, and residual error was used as the error term for testing the sample date and treatment × sample date interaction effects. Separate analyses were performed on the data for each year because different sets of treatments were evaluated in each of the 3 years. The main experiment (continuing treatments) and the sub-experiment initiated in 1998, which consisted of treatments to evaluate effects of withholding manure and fertilizer (residual fertility), were analyzed separately. Main-factor means were compared using Tukey’s Honest Significant Difference (HSD) calculated with $P = 0.05$ (Snedecor and Cochran, 1980). Fisher’s Least Significant Difference (LSD), calculated with $P = 0.05$, was used as an indication of variation in graphs showing temporal dynamics (Figs. 1, 2).

**Results and Discussion**

Sustained use of dairy manure slurry for 4 to 6 years increased *P. penetrans* population densities relative to the nontreated control (Table 1; Fig. 1). Main-factor effects of treatment and sample date, but not the treatment × date interaction, were significant in all 3 years (Table 1). *Pratylenchus penetrans* population densities in the high fertilizer treatment were generally intermediate to the control and high manure treatments but differed only from the control ($P \leq 0.05$) in 1999 and from the high manure treatment in 1997 ($P \leq 0.05$) (Table 1; Fig. 1).

Our results contrast with many previous studies indicating that manures, composted manures, and biosolids can reduce populations of plant-parasitic nematodes (Abawi and Widmer, 2000; Bulluck et al., 2002; Castagnone-Sereno and Kermarrec, 1991; Conn and Lazarovits, 1999; Kaplan and Noe, 1993; Kaplan et al., 1992; Oka and Yermiyahu, 2002; Riegel and Noe, 2000; Riegel et al., 1996; Stirling et al., 1995). The nematode-suppressive effects of manures and biosolids have often been attributed to ammonia (NH$_3$) toxicity (e.g., Castagnone-Sereno and Kermarrec, 1991; Conn and Lazarovits, 1999; Oka and Yermiyahu, 2002; Rodriguez-Kabana and Morgan-Jones, 1987), although there is also speculation that microbial antagonists of nematodes proliferating in manured soil may play a role in nematode suppression (Kaplan et al., 1992; Riegel and Noe, 2000; Riegel et al., 1996). Nitrous acid and volatile fatty acids have also been implicated in mortality of soilborne fungal pathogens after application of N-rich amendments (Tenuta and Lazarovits, 2002; Tenuta et al., 2002). Previous studies demonstrating nematode suppression generally involved incorporation of relatively large amounts of materials into soil prior to planting annual field, vegetable, or greenhouse crops or before planting a perennial horticultural crop. For example, Conn and Lazarovits (1999) suppressed *P. penetrans* under potato with pre-plant application of poultry manure at 1,573 kg total N/ha or 460 kg NH$_4^+$ N/ha. Our study differed because the manure was applied 2 to 4 times/year to grass, each time at approximately 100 kg NH$_4^+$N ha$^{-1}$, which may have been insufficient to generate toxic levels of NH$_3$, volatile fatty acids, or nitrous acid in the soil profile. Soil pH under all treatments was less than 5.9 (Bittman, unpubl. data), which would have favored formation of NH$_4^+$ relative to the more toxic NH$_3$. Finally, rapid uptake of N by the continuous grass cover and large microbial biomass supported by high root C inputs (Bittman et al., 2005) may have further reduced the potential for nematode-toxic concentrations of NH$_3$, volatile fatty acids, or nitrous acid to develop. Even if reduction of soilborne pathogens is a primary objective, manures should not be applied in excess of crop N requirements.
In our study, sustained use of manure increased not only *P. penetrans* population densities but also forage yields (Bittman et al., 2004). Although *Pratylenchus* spp. can have negative effects on stands of fescue (Cook and Yeates, 1993; Thies et al., 1995), relationships between nematode population densities and yield are not sufficiently well understood to estimate the degree of grass growth suppression associated with the *P. penetrans* population densities we observed in this study. Furthermore, earlier reports of relationships between tall fescue and *Pratylenchus* spp. are difficult to interpret because the authors did not report whether the fescue populations were infected with the endophyte *Neotyphodium coenophialum*, which confers resistance to plant-parasitic nematodes (Kimmons et al., 1990). Non-endophyte varieties of tall fescue are generally used for forage production in coastal British Columbia as well as in our study, specifically.

The high manure plots had lower stand densities (more bare soil) than the other treatments (Bittman et al., 1999b). This effect was likely the result of manure applicator disturbance or intra-specific competition for nutrients causing productive individuals to grow tall and shade less productive individuals. Our observations suggest that increased population densities of *P. penetrans* in manured plots, reducing growth of less resistant plants within the fescue population, also could have contributed to this effect. In any case, any incremental damage caused by increased *P. penetrans* populations was not sufficient to offset the benefits of increased nutrient availability associated with use of manure. The efficiency of nutrient capture by forage crops generally declines at higher nutrient loading rates (Bittman et al., 1999b), and our observations have led us to hypothesize that fescue response to manure, and the efficiency of nutrient uptake from manure, may have been greater at a site without *P. penetrans*. White clover, a common associate of fescue in these swards, is susceptible to and intolerant of *P. penetrans* (Cook and Yeates, 1993) and often does not persist with fescue when N availability is high (Bittman et al., 1999b). Willis (1980) reported that red clover establishment in Prince Edward Island was inhibited by *P. penetrans*. In our study, white clover volunteered in control plots but not in manured plots (Bittman, pers. obs.), and we hypothesize that buildup of *P. penetrans* populations on fescue may contribute to the poor volunteer establishment of white clover in heavily fertilized or manured soil.

The greater *P. penetrans* populations under manure-treated fescue could have implications for subsequent...
The mechanisms leading to increased *P. penetrans* population densities in manured and fertilized soil are not clear and cannot be determined from our field experiment. Possibilities include increased fecundity of nematodes feeding on nutrient-enriched roots, increased root growth providing more feeding sites, and suppression of natural biological control. Nutrient-enriched plants generally support greater populations of herbivores (Mattson, 1980), and other studies have reported increased population densities of plant-parasitic nematodes in N-fertilized plots (Dmoska and Ilieva, 1995; Sarathchandra et al., 2001; Todd, 1996; Yeates, 1987). We did not collect data on root N, but herbage N concentration in the high fertilizer treatment was significantly greater than in the high manure treatment (27% vs. 25%, respectively) (Bittman et al., 2004). However, the latter treatment harbored greater *P. penetrans* population densities. In addition, when compared on the basis of total N inputs (low manure vs. high fertilizer treatment) *P. penetrans* populations were greater (*P* ≤ 0.05) in manured soil than in fertilized soil in 1 of the 2 years compared. Average annual herbage yield was significantly greater in the high manure treatment than in the high fertilizer treatment (15 t (m)/ha vs. 13.4 t (m)/ha, respectively) (Bittman et al., 2004). Assuming root biomass parallels herbage biomass, then increased feeding sites appears to be a more likely explanation for the high *P. penetrans* abundance in manured soil than increased tissue N concentration.

High inputs of N-rich and labile organic residues are a type of disturbance to soil microbial communities, altering microbial and nematode community structure and potentially reducing the activity of antagonists of plant-parasitic nematodes, such as nematode-trapping fungi, endoparasitic fungi, or predacious nematodes. Jaffee et al. (1994) found that addition of poultry manure compost reduced infection of *Criconemella xenoplax* by the endoparasitic fungus *Hirsutella rhossiliensis*, and speculated that the *H. rhossiliensis* may have been negatively affected by ammonia from the compost whereas *C. xenoplax* was not. Although nematode-trapping and endoparasitic fungi were abundant in our field plots, we did not quantify differences among treatments. Omnivorous and predacious nematodes were reduced (*P* ≤ 0.05) in manure and fertilizer treatments relative to the control in all 3 years (Forge et al., 2005). This observation is consistent with the hypothesis that manure increased population densities of *P. penetrans* by reducing populations of antagonists or predators, but it does not rule out alternative hypotheses. Further, attempts to correlate populations of *P. penetrans* with populations of omnivorous and predacious nematodes across plots (rather than treatment means) were inconsistent. Coefficients of correlation between *P. penetrans* and predacious or predacious + omnivorous nematodes were 0.22 or 0.37 in 1998, respectively, and 0.23 or 0.13 in 1999, respectively.

### Table 1. Main-factor means for effects of manure and fertilizer treatments on population densities of *Pratylenchus penetrans* in 1997, 1998, and 1999. High treatments were 100 kg NH₃-N/ha/application, and low treatments were 50 kg NH₃-N/ha/application.

<table>
<thead>
<tr>
<th>Pratylenchus penetrans</th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
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<tbody>
<tr>
<td><strong>Continuous treatments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>211 B</td>
<td>185 C</td>
<td>206 C</td>
</tr>
<tr>
<td>High manure</td>
<td>341 A</td>
<td>326 AB</td>
<td>503 A</td>
</tr>
<tr>
<td>High fertilizer</td>
<td>218 B</td>
<td>196 BC</td>
<td>363 AB</td>
</tr>
<tr>
<td>Low manure</td>
<td>397 A</td>
<td>314 BC</td>
<td>503 A</td>
</tr>
<tr>
<td>Low fertilizer</td>
<td>341 A</td>
<td>326 AB</td>
<td>503 A</td>
</tr>
<tr>
<td>HSD (Trt)</td>
<td>97</td>
<td>130</td>
<td>147</td>
</tr>
<tr>
<td><em>P</em>-value (Trt)</td>
<td>0.045</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>P</em>-value (Date)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td><em>P</em>-value (Int)</td>
<td>0.778</td>
<td>0.082</td>
<td>0.503</td>
</tr>
<tr>
<td><strong>Residual fertility</strong></td>
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<tr>
<td>1994–1997 treatments</td>
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</tr>
<tr>
<td>High manure</td>
<td>323 A</td>
<td></td>
<td>522</td>
</tr>
<tr>
<td>High fertilizer</td>
<td>151 B</td>
<td></td>
<td>360</td>
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<tr>
<td>HSD (Trt)</td>
<td>66</td>
<td>93</td>
<td></td>
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<tr>
<td><em>P</em>-value (Trt)</td>
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<td>0.135</td>
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<tr>
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<td>0.001</td>
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<tr>
<td><em>P</em>-value (Int)</td>
<td>0.211</td>
<td>0.003</td>
<td></td>
</tr>
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</table>

*Values within a column and analysis group labeled with the same letter are not significantly different (Tukey’s HSD, *P* < 0.05).  
*HSD* (*P* = 0.05) values for main-factor effects of fertility treatments (*n* = 20, 24, and 32 in 1997, 1998, and 1999, respectively).  
* *P*-values for effects of the treatment (Trt), sample date (Date), and treatment × date interaction (Int). Data were analyzed using split-plot ANOVA models with treatments as whole-plots and dates as sub-plot factors. Separate analyses were conducted for each year and for the main experiment (Continuing treatments) and the sub-experiment to evaluate effects of withholding manure or fertilizer from previously treated plots (Residual fertility).  
n.a. = not applicable.
In summary, we found that sustained use of dairy manure slurry increased population densities of *P. penetrans* under tall fescue. These results contrast with many previous studies demonstrating decreased population densities of plant-parasitic nematodes in manure-treated soil and suggest that some care should be taken in recommending the use of manure for nematode management. We speculate that frequent applications of moderate amounts of manure to a perennial grass crop obviated the development of nematode-toxic concentrations of NH₃ or other toxic substances such as nitrous acid or volatile fatty acids. Nitrogen inputs alone could not explain the increase in *P. penetrans* population densities in manured soil, as applications of inorganic N fertilizer at rates comparable to the NH₃-N and total-N in manure treatments did not increase *P. penetrans* population densities to the same levels as in manured soil. Elucidating the mechanisms for increased population densities in soil treated with dairy manure slurry would require more detailed study of the effects of dairy manure on nematode antagonists and changes in host physiology. There was no clear relationship between changes in the abundance of *P. penetrans* and changes in the abundance of omnivorous and predacious nematodes.

**Literature Cited**


