NEMATODE DENSITIES IN YEAR-ROUND FORAGE PRODUCTION SYSTEMS UTILIZING MANURE FERTILIZATION

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ABSTRACT

Timper, P., G. L. Newton, A. W. Johnson, and G. J. Gascho. 2004. Nematode densities in year-round forage production systems utilizing manure fertilization. Nematropica 34:219-227.

Application of dairy manure to forage crops is a viable means of recycling nutrients and minimizing problems with manure storage. The objective of this study was to determine whether forage cropping system or frequent application of liquid dairy manure affected populations of plant-parasitic nematodes. Two forage systems were evaluated: the CBR system was a rotation of temperate corn (*Zea mays* L.), coastal bermudagrass [*Cynodon dactylon* (L.) Pers.], and a mixture of rye (*Secale cereale* L.) and crimson clover (*Trifolium incarnatum* L.); and the CCR system was a rotation of temperate corn, tropical corn, and a mixture of rye and crimson clover. Application of liquid manure at 14-day intervals with irrigation water was compared to application of inorganic fertilizer based on crop recommendations. The soil was naturally infested with *Helicotylenchus* sp., *Mesocriconema* sp., *Paratrichodorus* sp., and *Pratylenchus* spp. Densities of *Mesocriconema* sp. and *Helicotylenchus* sp. tended to be greater in CBR plots than in CCR plots, while the reverse was observed for *Pratylenchus* spp. Densities of *Paratrichodorus* sp. and *Pratylenchus* spp. were consistently lower in the manure treatment than in the fertilizer treatment. When averaged across years, numbers per 150 cm^3 of soil in manure vs. fertilizer were 79 vs. 159 for *Pratylenchus* spp. and 8 vs. 16 for *Paratrichodorus* sp. The other plant-parasitic nematodes were unaffected or inconsistently affected by nutrient source. Additional research is needed to determine the mechanism of nematode suppression when liquid dairy manure is applied regularly to the crop. If ammonia accumulation is involved, then soil type and pH will be important factors in determining the efficacy of the treatment.

Keywords: Bermudagrass, corn, cropping system, manure, nematode suppression, plant-parasitic nematodes, *Zea mays.*

RESUMEN

Timper, P., G. L. Newton, A. W. Johnson, y G. J. Gascho. 2004. Densidades de nemátodos en sistemas de producción continua de forraje utilizando fertilización de estiércol. Nematropica 34:219-227.

Aplicación de estiércol de lechería a cultivos de forraje es un método viable para reciclar nutrientes y minimizar problemas de almacenamiento de estiércol. El objetivo de este estudio fue determinar si el sistema de cultivo de forraje o la aplicación frecuente de estiércol líquido de lechería afecta las populaciones de nemátodos parásitos de plantas. Se evaluaron dos sistemas de forraje: el sistema CBR era una rotación de maíz templado (*Zea mays* L.), pasto Bermuda costeño [*Cynodon dactylon* (L.) Pers.], y una mezcla de centeno (*Secale cereale* L.) y trébol caramesi (*Trifolium incarnatum* L.); y el sistema CCR era una rotación de maíz templado, maíz tropical, y una mezcla de centeno y trébol caramesi. Aplicación de estiércol líquido a intervalos de 14 días con agua de riego fue comparada con aplicación de fertilizante inorgánico basada en las recomendaciones para el cultivo. El suelo era naturalmente infestado con *Helicotylenchus* sp., *Mesocriconema* sp., *Paratrichodorus* sp. y *Pratylenchus* spp. Densidades de *Mesocriconema* sp. y *Helicotylenchus* sp. tenían la tendencia de ser mas altas en parcelas de CBR que de CCR, mientras que lo opuesto fue observado para *Pratylenchus* spp. Densidades de *Paratrichodorus* sp. y *Pratylenchus* spp. eran consistentemente más bajas en los tratamientos con estiércol que con fertilizante. Tomando el promedio de varios años, los números por 150 cm^3 de suelo en tratamiento de estiércol comparado con fertilizante eran 79 con 159 for *Pratylenchus* spp. y 8 con 16 for *Paratrichodorus* sp., respectivamente. Los otros nemátodos parásitos de plantas no fueron afectados o fueron inconsistentemente afectados por la fuente de nutrientes. Se necesitan investigaciones adicionales para determinar el mecanismo de supresión de nemátodos cuando estiércol se aplica regularmente al cultivo. Si la acumulación de amonio es parte del efecto, entonces el tipo de suelo y pH serán factores importantes que pueden determinar la eficacia del tratamiento.

Palabras clave: estiércol, maíz; nematodos parásitos de plantas, Pasto Bermuda, sistema de cultivo, supresión de nemátodos, *Zea mays*.

INTRODUCTION

Dairy, livestock, and poultry production has been concentrated into units containing high animal densities, often on land with limited acreage or suitability for manure distribution (Pagano and Abdalla, 1994). In the southern United States, confined dairy cattle produce about 13 billion kg of manure per year which contains almost 7 million kg of nitrogen and over 1 million kg of phosphorus (Kellogg *et al.*, 2000). Storage and application of animal manure leads to problems with nutrient loss, odor, and contamination of surface and groundwater (Gascho, 2002). Although application of manures to forage crops is a viable means of recycling nutrients and minimizing problems with manure storage, when the amount of nutrients applied exceeds crop utilization, mobile nutrients such as nitrate can be leached into groundwater (Hubbard *et al.*, 1987; Sewell, 1975). Likewise, phosphorous and other nutrients, when applied in excess, can run off as particulate matter and contribute to eutrophication of surface water (Moore *et al.*, 1995). Frequent application of liquid manure in a year-round forage production system should maximize nutrient utilization by the crops and minimize losses to the environment.

Several studies have demonstrated suppression of plant-parasitic nematodes by the addition of animal manures including chicken litter (Conn and Lazarovits, 1999; Gonzalez and Canto-Saenz, 1993; Kaplan and Noe, 1993; Riegel and Noe, 2000; Riegel *et al.*, 1996), swine manure (Conn and Lazarovits, 1999), and composted mixtures of animal and plant material (Akhtar, 2000; Akhtar and Mahmood, 1996); however, other studies were unable to document nematode suppression. In most of these cases the animal manure had a neutral effect on nematode numbers (Akhtar, 1998; Ijani *et al.*, 2000; Sipes *et al.*, 1999), but a few studies have shown an increase in nematode numbers (Kimpinski *et al.*, 2003; Neher and Olson, 1999). In all of the above cited studies, the manure was incorporated in relatively large amounts into the soil before planting. The effect of frequent, year-round applications of manure on nematode populations has not been studied.

This research was part of a larger study to evaluate the efficiency of year-round forage production systems for utilizing dairy manure (Newton *et al.*, 2003; Newton *et al.*, 2000). Two forage systems were evaluated: the CBR system was a rotation of temperate corn (*Zea mays* L.), coastal bermudagrass [*Cynodon dactylon* (L.) Pers.], and a mixture of rye (*Secale cereale* L.) and crimson clover (*Trifolium incarnatum* L.); and the CCR system was a rotation of temperate corn, tropical corn, and a mixture of rye and crimson clover. Liquid manure, applied with sprinkler irrigation water, was compared to inorganic fertilizer application. The specific objective of this portion of the study was to determine whether cropping system or frequent application of liquid dairy manure affected population densities of plant-parasitic nematodes.

MATERIALS AND METHODS

The experiment was initiated in 1997 on a Tifton loamy sand (89% sand, 0% silt, 11% clay; pH 5.6) at the Animal Science Farm in Tifton, GA. Twelve 10-m × 10-m plots (five beds wide) were serviced by a traveling irrigation simulator that was capable of applying liquid manure and fertilizer. The experiment was a randomized, complete block design with a two-way factorial arrangement of treatments. The two factors were forage system (CBR and CCR) and nutrient source (liquid dairy manure and inorganic fertilizer). Each treatment combination was replicated three times. The experiment was concluded at the end of 2001.

For the CBR cropping system, temperate corn ('AgraTech 9250', 'AgraTech 999' or 'DeKalb 743') was no-till planted at 21 kg/ha into rye stubble and 'Tifton 44' coastal bermudagrass sod between the last week in March and the first week of April. The corn forage was harvested in mid-July. The bermudagrass continued to grow as an understory of the corn throughout the summer. In mid-November, a mixture of 'Wrens Abruzzi' rye and 'Dixie' crimson clover was planted into the bermudagrass sod using a no-till drill. The seeding rate of the mixture was 126 kg/ha rye and 9 kg/ha clover.

For the CCR cropping system, temperate corn was planted and harvested at the same time as in the CBR cropping system. Tropical corn (*Z. mays*, 'Pioneer X304-C' or 'Pioneer 3098') was planted at 23 kg/ha after harvest of the temperate corn. The tropical corn was harvested between late October and mid-November. Between late November and mid-December, a mixture of 'Abruzzi' rye and crimson clover was planted (15 to 37 days later than for CBR) into the corn stubble using a no-till drill. Rhizobium inoculum was applied to the clover in the fertilizer treatment but not in the manure treatment in both cropping systems. Insecticides, herbicides, and fungicides were applied according to crop recommendations for Georgia (Delaplane, 1996).

The liquid dairy manure was applied at approximately 14-day intervals for a projected total of 600 kg nitrogen/ha/year. The manure was applied with sprinkler irrigation on consecutive days, except when delayed by rainfall. When rain delays occurred, attempts were made to make up for any missed applications at a later time without exceeding 2.54 cm equivalents of water at any one time. There were between 23 and 25 applications of manure per year. The inorganic fertilizer was applied based on soil tests and recommended rates of N-P-K for the corn, bermudagrass, and rye crops (Plank, 1989). In 1997 and 1998, liquid fertilizer (10-10-10, N-P-K) was injected into the irrigation water the day after planting temperate corn to supply the recommended amount of potassium and phosphorus for the crop along with some nitrogen. In 1998, additional potassium (muriate of potash) was also applied with a yard spreader 2 days after planting. In 1999 to 2001, a liquid starter fertilizer (10-34-0, N-P-K) was banded beside the row during the spring corn planting to supply the same nutrient needs as described above. For the other crops, potassium (muriate of potash) and lime (dolomitic) were applied near planting time when soil tests indicated a need. Additional nitrogen applications were made by injecting a urea-nitrate solution (28% N, 5% sulfur) into the irrigation water. This was done four to five times for both temperate and tropical corn, twice for bermudagrass, and three to four times for the rye/clover crop.

Soil samples for nematode densities were collected in April and September. The samples consisted of 10 soil cores (2.5-cm diam. × 25-cm deep) collected from the inner side of beds one and two, and 10 from the inner side of beds four and five. The sample was thoroughly mixed and nematodes were extracted from a 150-cm^3 subsample by centrifugal flotation (Jenkins, 1964).

The effects of year, crop system, and nutrition on nematode densities in April and September were determined by analysis of variance (SAS Institute, Cary, NC). All two-way interactions of the three factors were included in the model. Differences among means were determined with Fisher's LSD test.

RESULTS

The mean cumulative amount of nitrogen applied to the plots yearly was 615 kg/ha for the CBR-manure plots, 611 kg/ha for the CCR-manure plots, 429 kg/ha for the CBR-fertilizer plots, and 425 kg/ha for the CCR-fertilizer. Soil pH fluctuated between 5.2 and 5.5 in the fertilizer plots, but steadily increased in the manure plots from 5.6 in January 1997 to 6.9 in November 2001.

Four genera of plant-parasitic nematodes were found at the field site: *Helicotylenchus*, *Mesocriconema*, *Paratrichodorus*, and *Pratylenchus*. Only *Pratylenchus* was identified to species. *Pratylenchus zeae* Graham and *P. scribneri* Steiner were present in a mixed population with the former being the dominant species (Z. Handoo, pers. comm.). Densities of individual genera were variable from year to year (Table 1). Nematode densities tended to be lower in 1997 and 1998 and higher in 1999 to 2001, particularly *Pratylenchus* spp. and *Mesocriconema* sp.

In the September samples, cropping system affected *Helicotylenchus* sp. ($P = 0.006$), *Mesocriconema* sp. ($P = 0.05$), and *Pratylen-*

Table 1. Number of nematodes of each genus in 150 cm^3 of soil collected in September of each year from a corn-corn-rye (CCR) and a corn-bermudagrass-rye (CBR) year-round cropping system.

Nematode	Crop system[a]	Year				
		1997	1998	1999	2000	2001
Helicotylenchus sp.	CCR	0	1	5	8	1
	CBR	0	2	17	42*[b]	42*
Mesocriconema sp.	CCR	234	241	308	536	328
	CBR	176	216	318	582	911*
Paratrichodorus sp.	CCR	10	5	28	9	0
	CBR	21	3	34	11	0
Pratylenchus spp.[c]	CCR	8	9	526	123	195
	CBR	12	11	49*	86	170

[a]When the soil samples were collected in September, tropical corn was growing in the CCR plots and coastal bermudagrass was growing in the CBR plots.

[b]Values are the mean of three replicates each from manure and fertilizer treatments (N = 6). For each nematode within a column, means followed by an asterisk indicates that nematode densities differed ($P < 0.05$) in CCR and CBR plots.

[c]There was a mixed population of *Pratylenchus zeae* and *P. scribneri* with the former being the dominant species. The other plant-parasitic genera were not identified to species.

chus spp. (P = 0.0003); however, the direction of the effect differed among nematode genera (Table 1). For example, densities of *Mesocriconema* sp. and *Helicotylenchus* sp. were greater in CBR plots than in CCR plots in some years, while the reverse was observed for *Pratylenchus* spp. The effect of cropping system on nematode densities was not consistent among years (year × crop system interaction; $P < 0.05$). With most nematode genera, the effect of cropping system was stronger in September than in April. The exception was *Pratylenchus* spp. where densities in the April sampling date were greater in CCR than in CBR plots in 1999, 2000, and 2001 compared to the September sampling date where these same trends were different only in 1999 (Fig. 1).

There was an effect of nutrition source on densities of *Mesocriconema* sp. (P = 0.003), *Paratrichodorus* sp. (P = 0.05), and *Pratylenchus* spp. (P = 0.006) in September but not in April. With *Mesocriconema* sp., the effect was not consistent among years (year × nutrient interaction; $P < 0.0001$). For example, the fertilizer treatment had greater ($P < 0.05$) densities of this nematode than did the manure treatment in 1998 (306 vs. 151/150 cm^3), but had lesser ($P < 0.05$) densities than did the manure treatment in 2001 (271 vs. 968/150 cm^3). In other years, nutrient source had no effect on *Mesocriconema* sp. With *Paratrichodorus* sp. and *Pratylenchus* spp., densities were consistently lower in the manure treatment than in the fertilizer treatment (Fig. 2). When averaged across years, numbers/150 cm^3 of soil in manure vs. fertilizer were 79 vs. 159 for *Pratylenchus* spp. and 8 vs. 16 for *Paratrichodorus* sp.

DISCUSSION

Of the four genera of plant-parasitic nematodes present at the field site, *Pratylenchus* and *Paratrichodorus* are significant

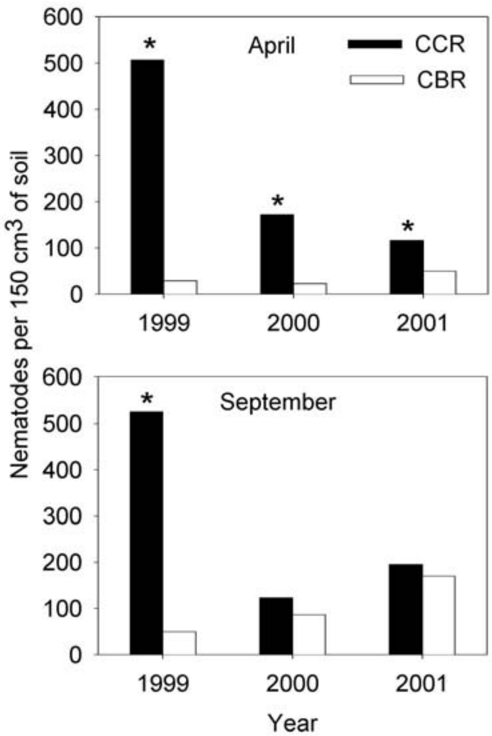

Fig. 1. Soil densities of *Pratylenchus* spp. in April (top graph) and September (bottom graph) under two different cropping systems: corn-corn-rye/clover (CCR) and corn-bermudagrass-rye/clover (CBR). Bars are the means of two nutrient treatments and three replicates (N = 6). An asterisk indicates that nematode densities differed ($P < 0.05$) in CCR and CBR plots in a given year.

pathogens of corn, while *Helicotylenchus* and *Mesocriconema* are considered moderate to weak pathogens of corn (Windham, 1998). Among the plant-parasitic nematodes present, only *Pratylenchus* spp. were above the damage threshold for corn (>300/150 cm^3) in CCR plots treated with fertilizer in September 1999 (Davis *et al.*, 1996). The higher soil densities of *Pratylenchus* spp. in the CCR compared to the CBR cropping system were probably due to the double crop of corn, which is a good host of *P. zeae* and *P. scribneri* (Endo, 1959; Rich *et al.*, 1977). Coastal bermudagrass appears

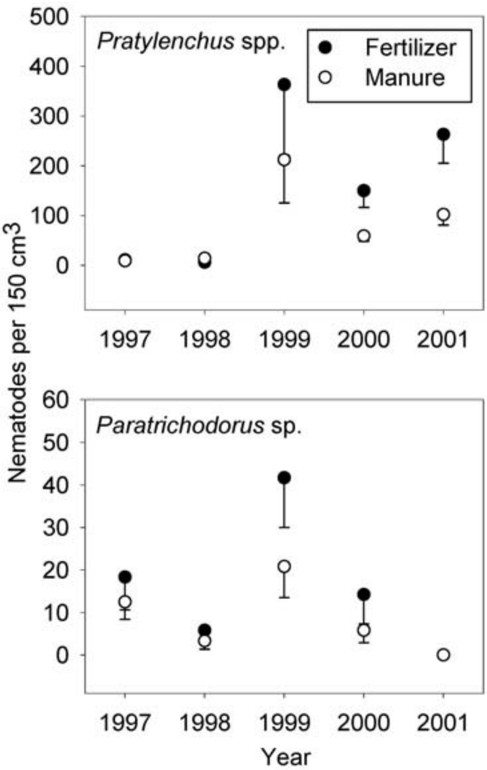

Fig. 2. Soil densities of *Pratylenchus* spp. and *Paratrichodorus* sp. in plots treated with conventional fertilizer and liquid manure (applied at ca. 14-day intervals). Densities of both nematodes were lower ($P < 0.05$) in manure than in fertilizer plots. There was no interaction between year and nutrient source. Data points are the means of two cropping systems and three replications (N = 6).

to be a poor host for *P. zeae*, the dominant *Pratylenchus* species at the field site. Brodie *et al.* (1969) showed that densities of this nematode were lowest in continuous bermudagrass, intermediate in corn following 3 years of bermudagrass, and highest in a continuous corn-rye cropping system. The differences in soil densities of *Pratylenchus* spp. between the CCR and CBR systems were larger in April than in September. Perhaps a greater proportion of the nematode population was within the roots of corn than bermudagrass in September.

During the growing season, most individuals of *Pratylenchus* sp. are within the roots. At the end of the season when the roots begin to die, a higher proportion migrate into the soil (MacGuidwin, 1989). However, much of the population will remain within the dead roots. Therefore, to accurately assess populations of *Pratylenchus* sp., both soil and roots should be sampled. Because only soil was sampled for nematodes in this study, we underestimated the population of *Pratylenchus* spp. The question is whether nematode densities from soil samples are proportional to the total population and reflect real treatment differences. For a given crop species, the proportion of *P. scribneri* outside the roots remained relatively constant during the growing season (MacGuidwin, 1989). Nitrogen deficiency can increase migration of *Pratylenchus* sp. out of roots (Patterson and Bergeson, 1967); however, in this study, nitrogen levels were optimum in all treatments. Differences in soil densities between manure and fertilizer treatments were consistent over cropping system and year. Therefore, it is likely that the lower soil densities of *Pratylenchus* spp. in the manure compared to the fertilizer treatment reflect differences in the total population of the nematode.

In this study, manure was applied to year-round cropping systems to recycle nutrients and reduce surface and groundwater contamination; it was not applied specifically to reduce numbers of plant-parasitic nematodes. Nevertheless, two nematode genera, *Pratylenchus* spp. and *Paratrichodorus*, were suppressed by the frequent application of relatively low concentrations of liquid manure during crop growth. In other studies, addition of cow manure (without added plant material) either had little effect on populations of plant-parasitic nematodes (Conn and Lazarovits, 1999) or increased nematode populations

(Kimpinski *et al.*, 2003; Neher and Olson, 1999). These conflicting results may be due to differences in physical, chemical, or microbial characteristics of the soils, or to the manner in which the manure was applied (at 14-day intervals with irrigation water vs. incorporated into soil before planting). Ammonia, which is generated during the decomposition of nitrogenous matter, is toxic to plant-parasitic nematodes (Rodriguez-Kabana, 1986). Soils with a low organic carbon and high sand content accumulate greater concentrations of ammonia than soils with a high organic carbon and low sand content (Lazarovits *et al.*, 2001; Tenuta and Lazarovits, 2002). The soil in our study was 89% sand whereas the soil was less than 70% sand in the other studies where manure failed to suppress nematode densities. Soil pH can also influence the ratio of ammonia:ammonium. In acidic soils, ammonia ionizes to ammonium and larger amounts of nitrogenous amendments are required to suppress nematode populations than are required in neutral or alkaline soils (Rodriguez-Kabana, 1986). The frequent application of manure increased the soil pH from 5.6 to 6.9 over the course of this study. The increase in soil pH likely led to greater ammonia concentrations and perhaps greater nematode suppression. Antagonistic microorganisms may also play a role in nematode suppression following addition of organic amendments (Boogert *et al.*, 1994; Jaffee, 2002; Linford *et al.*, 1938; Rodriguez-Kabana, 1986). Organic matter may either directly affect antagonists of nematodes by supporting growth of facultative saprophytes such as trapping fungi (Cooke, 1962) or indirectly affect antagonists by increasing densities of host nematodes such as bacterial feeders (Boogert *et al.*, 1994; Linford *et al.*, 1938). The addition of liquid manure at 14-day intervals may have maintained greater densities of nematode antagonists throughout the year than a one-time application of manure.

This is the first study demonstrating suppression of plant-parasitic nematodes by regular (14-day intervals) application of manure through irrigation water. Additional research is needed to determine whether the mechanism of suppression is the same as when large quantities of nitrogenous amendments are applied before planting. If ammonia accumulation is involved in nematode suppression when liquid manure is applied regularly to the crop, then soil type and pH will be important factors in determining the efficacy of the treatment.

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