

# Populations of *Pratylenchus penetrans* Relative to Decomposing Nitrogenous Soil Amendments<sup>1</sup>

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**Abstract:** Populations of *Pratylenchus penetrans* decreased in soil following addition of 70 and 700 ppm N in the form of nitrate, nitrite, organic nitrogen, or ammonium compounds. Nitrate was less effective than other nitrogen carriers. Population reduction is principally attributed to ammonification during decomposition. This hypothesis is supported by chromatographic analyses of soil atmospheres, survival of nematodes in pure CO<sub>2</sub> and N<sub>2</sub>, inverse relationship of CO<sub>2</sub> content in amended soils to nematode populations, and direct relationship of NH<sub>3</sub>-N content of amended soils to nematode populations. **Key Words:** Nitrogen amendments, Soil amendments, Decomposition, *Pratylenchus* populations, Ammonia, CO<sub>2</sub>, N<sub>2</sub>.

The decrease in plant parasitic nematode populations which often occurs after the addition of various organic soil amendments (5, 6, 10, 16, 17) has been attributed to predacious fungi (3), nematode-trapping fungi (12), toxins from fungi and/or higher plants (7, 12, 14), or changes in osmotic pressures within the soil environment (19). Little attention has been given to the natural breakdown processes (mineralization or immobilization) that might affect nematodes, although several investigators have suggested and presented some preliminary evidence that ammonia, as a product of decomposition, could be the nematicidal principle (4, 8, 9, 18).

The necessity of microbial activity for the effectiveness of an organic soil amendment (soybean meal) against *Pratylenchus penetrans* (Cobb) Filipjev & Shuurmans-Stekhoven has been demonstrated (21). A few of the common products of decomposition, particularly of nitrogen-containing substances have now been examined as they relate to lesion nematode populations. Part

of this investigation was published as an abstract (22).

## MATERIALS AND METHODS

Inorganic and organic nitrogen-containing substances were mixed at the rate of 70 and 700 ppm N (dry wt) with a sandy-loam greenhouse potting soil of pH 6.2. The soil had a mechanical analysis of 70% sand, 22% silt, and 8% clay and an ammonium nitrogen content equivalent to 34.5 kg/ha (76 lb./acre). The organic nitrogen materials used were peptone, soybean meal, skim milk, and urea. Inorganic nitrogen substances included (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>OH, KNO<sub>2</sub>, and NaNO<sub>2</sub>. Each chemical was mixed with 1000 g of air-dry soil, the moisture content adjusted to approximately 15% (oven dry-wt), and then 250 g soil samples of each treatment were placed into four 280 ml styrofoam cups. The open cups, covered loosely with a sheet of plastic, were incubated in a laboratory at 20–25 C. One day following treatment 1000 nematodes, in various developmental stages, from axenic corn callus cultures were pipetted into each cup. Twenty 5-cc soil samples were removed from each replicate cup after 1, 2, and 6 weeks, and assayed for nematodes with a soil sieve similar to that described by Yeates (23). This method proved most expedient for determining nematode populations. Previous

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investigations revealed that when nonmotile nematodes from SBM-treated soil were collected by screening then placed in small quantities of water, they did not become motile and were in the characteristic death position for this species. Nematode survival in treated soils was compared with survival in the nontreated (control) soils, and the data expressed as a percentage decrease or increase over the controls.

Amended and non-amended soils were analyzed for CO<sub>2</sub>, ammoniacal nitrogen, and for other major gaseous constituents by a mass spectrometer (Gallob Analytical Service, Inc., Berkeley Hts., New Jersey). A gas chromatographic analysis for several minor constituents present in the gaseous phase of amended and non-amended soils also was performed (Stadler Research Labs., Philadelphia, Pa.). These spectrometric and chromatographic analyses utilized samples incubated in serum-stoppered flasks containing 700 ppm N in the form of soybean meal (SBM) added to nonsterile soil. The samples were scanned from mass 2 through mass 100 with a detection threshold for most constituents from .001 to .01% after injecting 1 ml of the gas from the closed system into the gas chromatograph.

Ammoniacal nitrogen content in SBM, and urea-amended and non-amended soils was determined by the Kjeldahl procedure outlined by Prince (13). These determinations were made on 50 g (oven-dry wt) samples removed from polyethylene bags which were incubated for 2, 5, 9, and 16 days at ambient laboratory temperatures (20–25 C). Calculations of ammoniacal nitrogen (NH<sub>3</sub>-N) were based on titration with 0.02 N NaOH of the distillate collected in 0.02 N

HCl using methyl red-bromocresol green indicator.

Carbon dioxide production was measured on soil samples containing 700 ppm N of SBM, NH<sub>4</sub>NO<sub>3</sub>, urea, or KNO<sub>2</sub> by the bubbling tower method. Fifty ml of 1N NaOH was used in each collecting flask as the CO<sub>2</sub> absorbent. An excess of 3 N BaCl<sub>2</sub> was added to the NaOH prior to titration with 1N HCl. The air stream was bubbled through concentrated H<sub>2</sub>SO<sub>4</sub>, NaOH, and CO<sub>2</sub>-free water before entering the glass beaded columns of each unit. Determinations were made on replicate samples after 2, 3, 4, 7, 9, and 11 days incubation, and the data from each treatment averaged.

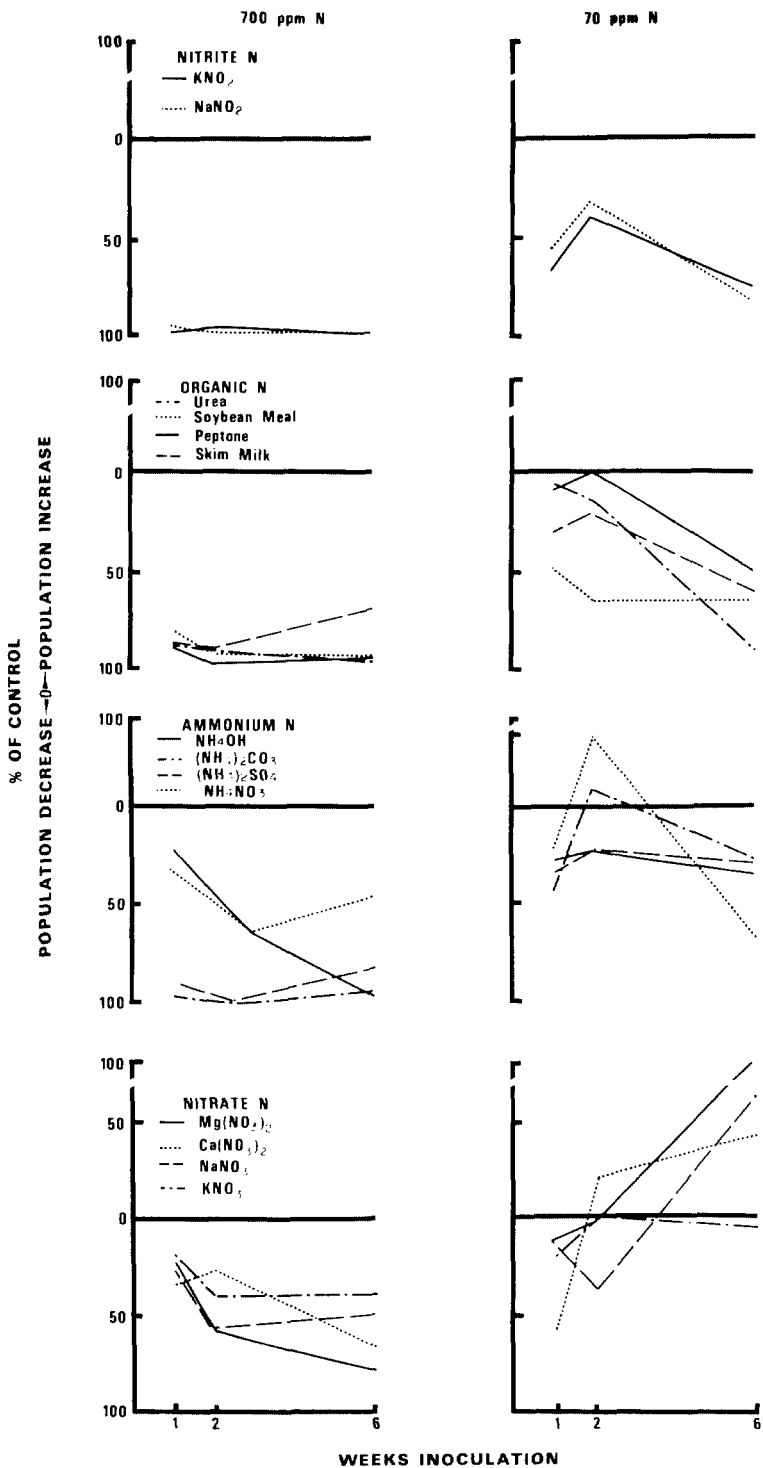
## RESULTS

**INFLUENCE OF NITROGENOUS SUBSTANCES ON *PRATYLENCHUS PENETRANS* POPULATIONS:** Nitrite, ammonium, and organic nitrogen sources were more detrimental to lesion nematode populations over a 6-week period than were nitrate compounds (Fig. 1). At 700 ppm, KNO<sub>2</sub> and NaNO<sub>2</sub> were lethal to the nematodes after 1 week. Organic nitrogen sources (SBM, urea, peptone, and skim milk) were less detrimental than nitrites, resulting in 75 to 90% population reduction after 1 and 2 weeks. Ammonium compounds varied in their effects during the first two weeks of incubation, but after 6 weeks had caused a population reduction comparable to that caused by the organic nitrogen sources.

Generally 70 ppm N caused less population reduction in 6 weeks than 700 ppm N. The nitrates at 70 ppm, however, caused an increase in the population compared with the controls. This greater number may have resulted from hatching from eggs; a response

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FIG. 1. Effect of nitrites, organic nitrogens, ammonium nitrogen, and nitrates on *Pratylenchus penetrans*.



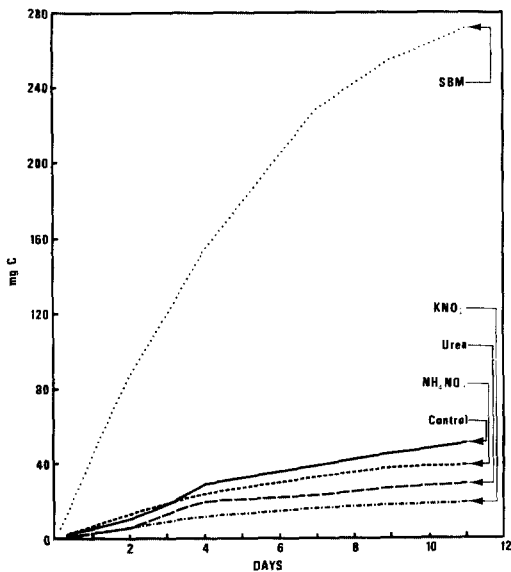


FIG. 2. CO<sub>2</sub> production in 700 ppm nitrogen-amended soil, expressed as mg accumulative carbon.

that either was masked or did not occur at the higher concentration of N.

**MASS SPECTROMETRIC AND GAS CHROMATOGRAPHIC ANALYSES:** Spectrometric analyses for the major constituents in the gaseous phases of nonsterile soil amended with 700 ppm N as SBM revealed that nitrogen and carbon dioxide comprised 58 and 41% (v/v) of gas in these samples. Other gases detected were: argon 0.64%, hydrogen 0.044% and oxygen 0.0052%. Interference by nitrogen prevented determination of carbon monoxide concentration. No ethylene was detected above 52 ppm and no methane above 20 ppm.

In addition to mass spectrographic analysis, the gaseous portions of a SBM-amended and non-amended soil samples were analyzed for ethylene and ammonia using a Perkin-Elmer 154-B vapor fractometer equipped with a column of synthetic polymer beads and a thermal conductivity detector. The operational conditions used for this analysis were: Column—10 ft. × ¼" aluminum tubing

packed with 80/100-mesh Porapak Q; Detector—Thermal conductivity; Carrier Gas—He @ 30 ml/min; Oven Temperature—152 C; 7 v d-c through the Weston Bridge. Recorded chromatograms showed three major peaks, identified by their retention characteristics as air, ethylene, and ammonia. Peaks of these gases were present in both amended and non-amended samples.

The elution retention time of the sample components was compared with the retention time of pure single components and of a mixture of ethylene and ammonia used as standards. A shift in retention time occurred with the soil samples when compared to that of ethylene and ammonia in the synthetic mixture. The presence of ammonia in the amended samples was confirmed by the blue positive reaction of a "precision" red litmus paper. Quantitation of the component gases on the chromatograms of the two samples was not possible because other asymmetric peaks were associated with the ammonia peak.

Inasmuch as ethylene was present in non-amended as well as amended samples and mass spectrographic analysis did not reveal ethylene above 52 ppm, further investigation on the influence of this gas or other short-chain hydrocarbons on nematode populations was not undertaken. Attention was directed instead to the other major components in the gaseous phase: carbon dioxide, nitrogen, and ammonia.

**CO<sub>2</sub> PRODUCTION IN NITROGENOUSLY AMENDED SOILS:** Production of CO<sub>2</sub> in soil amended with 700 ppm N (dry wt soil) of SBM, KNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>, and urea was compared with CO<sub>2</sub> production in non-amended soil. The accumulated CO<sub>2</sub> produced by SBM-amended soil exceeded that of any other nitrogenous amendment, and after 12 days was 5 times greater than the controls (Fig. 2). The least mg C was produced by KNO<sub>3</sub> amendment; greater amounts occurred

TABLE 1. Average number of nematodes recovered from 4 cylinders of soil after 2 weeks' exposure to air, carbon dioxide, and nitrogen.

Treatment	Experiment		
	1	2	3
Air	639	481	2,968
CO <sub>2</sub>	30	40	407
% Reduction	95	92	86
Air	3,962	1,827	1,565
N <sub>2</sub>	1,023	330	684
% Reduction	74	82	56

in the urea and NH<sub>4</sub>NO<sub>3</sub>-amended soils, however these did not exceed the mg C produced in the controls.

To further evaluate the significance of CO<sub>2</sub> on *Pratylenchus penetrans*, nematodes were exposed to commercial grade CO<sub>2</sub> for 2 weeks in Plexiglas® cylinders containing non-sterile soil according to previously described procedure (17). In 2 of 3 experiments 90% fewer nematodes were recovered from CO<sub>2</sub>-treated soil than from soil exposed to air (Table 1). It was assumed that the concentration of CO<sub>2</sub> in amended soils within styrofoam cups and polyethylene bags would vary, yet in both would be considerably less than the CO<sub>2</sub> atmosphere within the soil cylinders.

**NEMATODE POPULATIONS IN GASEOUS NITROGEN ATMOSPHERES:** Nematodes were exposed for 2 weeks to gaseous N<sub>2</sub> in the above soil cylinders. The average nematode population in three experiments using commercial grade N<sub>2</sub> was approximately 70 per cent less than the population similarly exposed to air for the same length of time (Table 1).

Although N<sub>2</sub> in soil cylinders was somewhat deleterious to *P. penetrans* populations after 2 weeks, the greater reduction in populations which resulted within 1 week in the open system (styrofoam cups), leads me to infer that nitrogen is not the primary principle responsible for decreased populations.

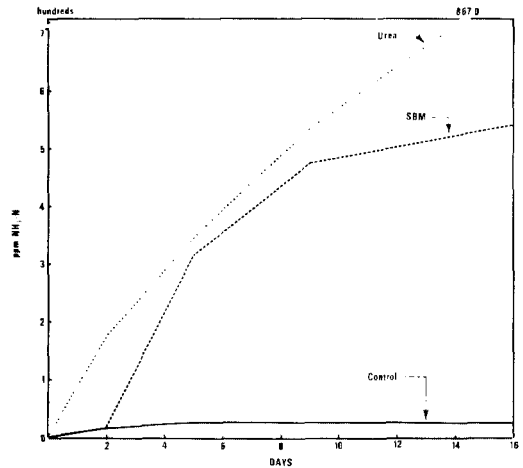


FIG. 3. Ammoniacal nitrogen production in nonamended soil and soil amended with 700 ppm N as urea or soybean meal (SBM).

**NH<sub>3</sub>-N CONCENTRATION OF AMENDED SOILS:** Ammoniacal N increased with incubation in SBM and urea-amended soils but not in the non-amended (control) soils (Fig. 3). All treatments resulted in NH<sub>3</sub>-N levels above 300 ppm and 500 ppm after 5 and 16 days, respectively. The highest concentration of NH<sub>3</sub>-N occurred in urea-treated soil; lesser amounts were produced by SBM, especially at 2 and 16 days. These same nitrogen sources, when added at 700 ppm N also decreased nematode populations within one week (Fig. 1).

## DISCUSSION

Compared with other nitrogen sources, nitrite caused the greatest reduction of lesion nematode populations during the first 6 weeks' incubation.

It is recognized that excessive concentrations of nitrite are injurious to cells. Results in previous studies suggest that KNO<sub>2</sub> may be directly toxic to lesion nematodes at 700 ppm N even under sterile conditions. After 6 weeks the effects of organic and ammonium nitrogens were comparable to nitrites.

Nitrate compounds, except for ammonium nitrate, were less effective than the others. Actually nematode populations in soils amended with 70 ppm nitrate generally were higher than the controls. These results confirm reports of a similar effect of certain nitrogenous compounds on nematode populations (5, 8).

The microbial breakdown of nitrogen-containing substances in soil via the processes of ammonification (mineralization) and nitrification, resulting in ammonia, ammonium ions, and subsequently nitrite and nitrates, would suggest that these products of decomposition might be as operative against nematodes as an increase in predacious nematodes, "nematode-trapping" fungi or their toxins.

The nematicidal effect of  $\text{NH}_3$  *per se* on plant parasitic nematodes was demonstrated in 1955 by Eno *et al.* (4). They found a sharp decrease in nematodes after field applications of 365 and 608 ppm ammoniacal nitrogen. In these experiments ammonia concentrations greater than 300 ppm were measured in soils within 5 days after amendments with SBM and urea. In previous studies (20), lesion nematodes in a droplet of water were killed after an exposure of 1 hr to 800 ppm  $\text{NH}_3$ . Birchfield and Parr (1) recently found that  $\text{NH}_3$  alone and  $\text{NH}_3$  formulated with  $\text{KN}_3$  (potassium azide) significantly reduced *Rotylenchulus reniformis* within a 5-cm zone surrounding the point of application.

The low oxygen concentration (.0052%) in the atmosphere of the closed system, as detected by spectographic means, might suggest that nematode motility could be affected by low oxygen in combination with other gases. However, the oxygen concentration in the open cups would be considerably greater. A further suggestion that oxygen was not a limiting factor occurred in several of the 700 ppm N experiments where nematodes were reintroduced into the cups 6 weeks after the initial infestation, and the soil surface stirred

daily. Nematicidal activity was still apparent 2 weeks later (author's unpublished data).

High concentrations of  $\text{CO}_2$  and  $\text{N}_2$ , singly or in combination, may have considerable influence on long term population shifts, yet the  $\text{CO}_2$  production in urea,  $\text{NH}_4\text{NO}_3$  and  $\text{KNO}_2$ -amended soils was inversely related to the decrease in nematode population. It is unlikely that lower  $\text{CO}_2$  concentrations found in amended soils is responsible for the marked population reduction associated with nitrogenous amendments in polyethylene bags or styrofoam cups since some nematodes survived 2 wks exposure to "high"  $\text{CO}_2$  concentrations.

I conclude that  $\text{NH}_3$  or possibly nitrite, produced during the decomposition of nitrogenous substances are among the principle compounds responsible for the decreased plant parasitic nematode populations often noted following organic amendment. The direct or indirect influence of pH,  $\text{Ca}^{+}$  ions, moisture (2), temperature, type of amendment (11, 15), and other factors on microbial activity, will affect nitrification and nematode populations. These variables may explain, to some degree, the results obtained with other amendments.

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