Pratylenchus penetrans (Cobb) Populations as Influenced by Microorganisms and Soil Amendments.[1]

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Abstract: Numbers of *Pratylenchus penetrans* in sterilized soil decreased significantly 2 weeks after the addition of 1% $^{w}/_{w}$ (700 ppm N) nonsterile soybean meal (SBM), or sterilized SBM in combination with selected microorganisms. Sterilized SBM had no effect on nematode populations in steamed soil. Bacteria and fungi in the presence of SBM were more effective than the actinomycetes tested, causing up to 96–100% reduction in nematode populations. Simpler nitrogenous compounds included KNO_2, $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2CO_3$, urea, and peptone, decreased nematode populations with variable effectiveness when added to steamed soil at 700 ppm N; KNO_2 was the most nematicidal.

Factors affecting the decline of plant-parasitic nematodes in soil during degradation of either complex organic material (2, 4, 7, 8) or simpler nitrogenous compounds (9) are not well understood, although presumably soil microorganisms are principally involved. Therefore, an understanding of the ecological relationships between nematodes and soil microbes should provide a sound basis for a practical program for biological control of plant-parasitic nematodes.

In 1967, Walker et al. (11) reported decreased populations of *Pratylenchus penetrans* (Cobb) Filipjev & Schurmans—Stekhoven in steamed soil following the addition of soybean meal. The experiments described below were designed to explore the cause of this nematicidal effect, particularly as it relates to specific microorganisms and the possibility that simpler nitrogenous compounds might also be detrimental to *P. penetrans*.

MATERIALS AND METHODS

In the previous investigations (11), steam sterilized soil was amended with 1% $^{w}/_{w}$ nonsterile soybean meal (SBM), infested with nematodes, and incubated in closed polyethylene bags for various lengths of time. Some of the same techniques, especially in the experiments with nonsterile SMB, were followed in the investigations reported here.

To determine the influence of this amendment on nematodes in the absence of microorganisms, SBM, available commercially as an animal feed containing 44% protein, was incorporated into a sandy-loam soil mix (1 : 3) at the rate of 1% (700 ppm nitrogen). The soil moisture was adjusted to 16–20% (dry weight). Two hundred forty ml of the amended soil was placed in each 500 ml Erlenmeyer flask and autoclaved at 15 psi for 50 min. The controls were treated similarly except SBM was omitted. Approximately 2000 nematodes from 3–6 month-old axenic nematode cultures on corn root callus (var. 'Longfellow Yellow Dent') were introduced aseptically to each replicate flask. The corn callus tissue was maintained on the medium suggested by Krusberg (3) and different age cultures were selected to provide nematodes of various developmental stages. Nematodes were extracted from the callus in a sterilized Baermann funnel; the funnel top was covered with a cotton layer held in place with a petri plate cover and a 50 ml cotton-stoppered flask was attached to the funnel outlet before autoclaving.

In all experiments the amended and non-amended soils were incubated at 23–27 C.

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After incubation, tubes of thioglycollate medium were inoculated with soil particles from each flask. Turbidity in these tubes after 5 days incubation at 37 C indicated contamination.

Following incubation, nematodes were recovered by placing 25 ml soil samples from each replicate on tissue in a soil sieve similar to that described by Yeates (12). The nematode populations were then compared with those recovered from the nonamended soils (controls).

To ascertain the influence of microbes on *P. penetrans* in the presence and absence of SBM, pure cultures of microorganisms were obtained by plating SBM on Difco nutrient agar. The predominant organisms isolated were bacteria and a *Fusarium* sp.; these were maintained and increased on nutrient agar. Inasmuch as the bacterial flora presumably would differ with different sources of SBM, the bacterial isolates were not identified in these studies. Sterile distilled water suspensions of microbes were prepared from 2–3 plates of each isolate and 5 ml aseptically added to sterilized sandy-loam soil contained in polyethylene bags or in 500 ml flasks. Each bag was sealed, shaken, and incubated for 1 week before infestation with nematodes. The nematode populations were determined as above after an additional 2 week's incubation. In the experiments utilizing flasks instead of plastic bags, all procedures were similar to the above, except to lessen the chances of contamination from repeated flask opening, microorganisms and nematodes were added simultaneously. Other experiments utilizing microorganisms were performed; the procedures applicable to these are presented in the appropriate section with the results.

The influence of simple nitrogenous substances on *P. penetrans* populations under sterile and nonsterile conditions was investigated. In the former, glucose was included as a carbohydrate source, separately and in combination with $NaNO_3$. Solutions of both substances were sterilized by Milipore® (0.45u) filtration and added aseptically at the rate of 200 mg nitrogen and 5 mg glucose/g dry wt. soil to 120 ml of autoclaved soil/flask. This glucose-nitrate concentration provided a low C : N ratio. One series of glucose-nitrogen amended soils was inoculated with mycelium-spore suspensions of the *Fusarium* sp. originally isolated from SBM. Another series was inoculated with a *Streptomyces* sp. (Isolate 581) whose cultural fluids are known to be moderately nematicidal (9). Nematode populations were determined after 1 week.

Other nitrogen sources were incorporated singly into 240 ml of sterilized sandy-loam soil containing 1000 nematodes in each of 4 plastic bags. The nitrogen sources were: SBM, KNO_2, $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2CO_3$, urea, and peptone. These were added at the rate of 700 ppm nitrogen. Nematode populations were determined after 1, 2, and 3 weeks.

RESULTS

Sterilized SBM in sterile soil caused no reduction of *P. penetrans* populations after two weeks of incubation when compared to nonamended soil; the percentage nematode recovery between amended and nonamended soils varied no more than 2%.

Conversely, nonsterile SBM in polyethylene bags of sterilized soil caused a 90% reduction in the nematode populations after 2 weeks (Table 1). When bacterial isolates obtained from SBM were reintroduced into amended soils, the population reduction was 96 to 100%. Two bacterial isolates (Nos. 1 and 4), when added to sterilized soil lacking the amendment, significantly reduced the populations below that of the control, but not as much as in the presence of SBM.

When sterile soil containing sterile SBM was inoculated with bacterial isolates, the

TABLE 1. Survival of *Pratylenchus penetrans* after 2 week's incubation in sterilized soils amended with nonsterile SBM.

Soil treatment	Nematodes recovered[a]	% reduction
No amendment	39	–
Soybean meal	4	90*
Bacterial isolate 1	21	47*
Bacterial isolate 2	35	9
Bacterial isolate 4	18	54*
SBM + isolate 1	1	97*
SBM + isolate 2	1	97*
SBM + isolate 4	0	100*
LSD (5%)	12	30

[a] Average of 4 replicates, 25 cc each. Original infestation level was approximately 200 nematodes/25 cc.
* Significant at the 0.05 level.

numbers of nematodes surviving were significantly less, in 2 of 3 experiments, than the number surviving in flasks containing sterile SBM alone (Table 2). Three of the 4 bacterial isolates were similar to each other in their effects on the nematode populations.

To determine if organisms other than those isolated from SBM might suppress nematode populations, two actinomycetes (*Streptomyces* sp., Isolates 1606 and 581) were separately introduced into sterile SBM-amended and nonamended sterile soil. Actinomycete inoculua were prepared from shake cultures incubated at 23–27 C on Difco Soytone medium for 3 days. The *Fusarium*

TABLE 2. *Pratylenchus penetrans* surviving in SBM-amended soil under sterile conditions 2 weeks following inoculation with bacterial isolates.

Soil treatment	Experiment 1	2	3
Soybean Meal (Sterile)	20[a]	26	15[b]
SBM + bacterial isolate 1	4*	1*	19
SBM + bacterial isolate 2	3*	6*	13
SBM + bacterial isolate 3	3*	5*	14
SBM + bacterial isolate 4	11*	13*	22
LSD (5%)	8	10	NS

[a] Average number of nematodes from 4 replicates, 25 cc each.
[b] Contamination in the controls and in several replicates within treatments was noted.
* Significant reduction at the 0.05 level.

TABLE 3. Survival of *Pratylenchus penetrans* in sterilized soybean meal (SBM) amended soils in presence and absence of selected microorganisms.

Soil treatment	recovered[a]	% reduction
No amendment	48	–
SBM (sterile)	45	6
Actinomycete #1606	13	73*
SBM + #1606	18	62*
Actinomycete #581	36	25
SBM + #581	75	0
Fusarium sp.	29	40
SBM + *Fusarium*	16	67*
LSD (5%)	23	48

[a] Average from 4 replicates, 25 cc each.
* Significant at the 0.05 level.

recovered from SBM was included for comparison. The fungus was grown on 50 g of sterile moistened wheat kernels incubated 7 days at 27 C. All microorganisms were separated from their cultural substrates by repeated washing with sterile water, followed by centrifugation in sterile tubes. Five ml of the resuspended inoculum were distributed to each flask.

Actinomycete isolate 1606 caused a significant decrease in populations either in the presence or absence of SBM (Table 3). Actinomycete isolate 581 in the absence of SBM had little effect on *P. penetrans* populations. With SBM supplement, a slight increase in populations (relative to controls) was noted. The *Fusarium* isolate caused a significant population reduction only in the presence of SBM.

Comparing the effects of glucose and $NaNO_3$ on nematode populations under sterile conditions, glucose alone had no apparent effect, whereas $NaNO_3$ alone or in combination with glucose, caused a significant decrease in nematode numbers after 1 week (Table 4). *Fusarium* caused a significant decrease in populations in the presence of SBM or $NaNO_3$, whereas actinomycete 581 was effective only in the presence of $NaNO_3$.

TABLE 4. Effect of SBM, glucose, and sodium nitrate amendments, singly and in combination with organisms, on *Pratylenchus penetrans* survival after 1-week incubation.

Soil amendment	Nematodes recovered[a]	% reduction
No treatment	53	–
SBM-sterile	61	0
Glucose[b]	53	0
Sodium nitrate[b]	10	81*
Glucose + $NaNO_3$	19	64*
Fusarium sp.	58	0
SBM + *Fusarium*	9	82*
Glucose + *Fusarium*	32	40
$NaNO_3$ + *Fusarium*	4	92*
Glucose + $NaNO_3$ + *Fusarium*	2	96*
Actinomycete #581	64	0
SBM + #581	43	19
Glucose + #581	89	0
$NaNO_3$ + #581	16	70*
Glucose + $NaNO_3$ + #581	14	74*
LSD (5%)	32	60

[a] Average from three 25 cc samples.
[b] Glucose added at the rate of 5 mg/g dry weight basis of soil; $NaNO_3$ at 200 ppm nitrogen/flask.
* Significant reduction at the 0.05 level.

The results with other simple nitrogen compounds on lesion nematodes can be summarized as follows. KNO_2 caused complete kill of nematodes within 1 week (Fig. 1). Over the 3 week period, the average nematode reduction with SBM and peptone was 90 and 86%, respectively. Urea caused only 21% reduction after 1 week, whereas the other nitrogen sources caused 64 to 84% reduction. After two weeks, urea diminished the populations by 60% and after 3 weeks by 85%. Ammonium carbonate, ammonium nitrate, and calcium nitrate were comparable in reducing nematodes over the 3-week test period, with average reductions of 74, 70, and 73%, respectively.

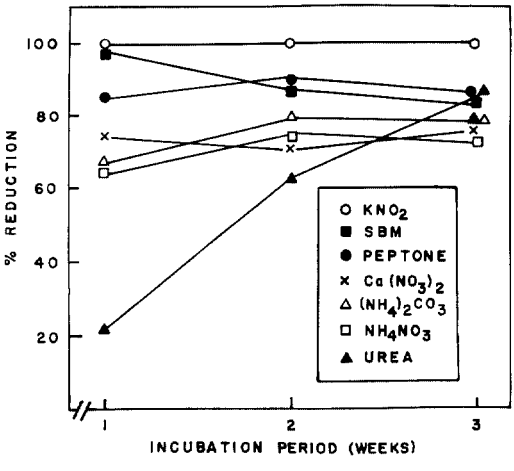

FIG. 1. Reduction of *P. penetrans* populations by different nitrogen sources at 700 ppm nitrogen.

DISCUSSION

To date, numerous amendments have been shown to be nematicidal to a variety of nematode species under laboratory, greenhouse and field conditions. Seldom has the significance of the microorganisms been demonstrated. Concommitant assays of microbial populations when undertaken generally have revealed an inverse relationship of microbial numbers to plant nematode populations (5). Since sterilized SBM under aseptic conditions did not suppress lesion nematode populations, we conclude that microorganisms and/or their degradation products are responsible for the decline of nematode populations after incorporation of this organic amendment. Fungi and bacteria produced greater nematicidal effect than actinomycetes. In previous studies, bacterial culture fluids were more nematicidal than those of actinomycetes (10).

Several reports indicate nitrogen fertilizer components are detrimental to nematodes (1, 6). Although the nitrogen level in our experiments was greater than that normally added to field soils, the results show that simple nitrogenous substances vary in toxicity to lesion nematodes at 700 ppm nitrogen. The results suggest that one benefit of high nitrogen fertilization might be reduction of plant parasitic nematode populations.

Although nematicidal metabolites originating directly from the microorganisms should be considered, nematode mortality in the

presence of decomposing high-N organic material, and certain simpler nitrogenous compounds, suggests an intermediate breakdown product during decomposition is responsible for reducing nematode populations.

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