Effects of Phenamiphos, Methyl Bromide, and Fallowing on *Pratylenchus penetrans*, Yield of *Medicago sativa*, and *Fusarium* Infections[1]

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Abstract: A field study was made of the effects of a residual nematicide (phenamiphos), a fumigant (methyl bromide), and fallowing on the number of root lesion nematodes (*Pratylenchus penetrans*), forage yields of alfalfa, and the occurrence of *Fusarium* spp. in plant roots and soil. Fallowing controlled nematodes initially, but by the end of the second growing season, nematode numbers were as high as in plots which had grown a nematode-susceptible crop. Forage yield was greater in fallowed plots only for the first cut in the year after seeding. *Fusarium* in alfalfa roots and soil was not reduced by fallowing. Phenamiphos reduced nematode numbers, increased forage yields in 2 of 4 years, and reduced *Fusarium* infections of taproots. Soil fumigation with methyl bromide gave the best control of nematodes and *Fusarium* and gave significantly higher forage yields for the 4 years of study following fumigation. The 34% increase in alfalfa yield from fumigated plots over the 4 years indicates that the yield of alfalfa is being reduced significantly by microorganisms. The study does not establish the relative contributions of the root lesion nematodes and *Fusarium* spp. to the reduction. *Key Words:* alfalfa, root lesion nematode, population dynamics, control, nematode-fungus-host interaction.

The root-lesion nematode, *Pratylenchus penetrans*, has been shown to reduce yields of alfalfa, *Medicago sativa* L., under greenhouse (8) and field (5, 6) conditions. The use of nematicides to control root lesion nematodes has increased forage yields. Coincident with reduced nematode numbers was reduced infection of root tissues by *Fusarium* spp. and other fungi even though laboratory tests indicated that the rates of the nematicides used in the field were not fungicidal (6). Soil sterilants such as methyl bromide have been used to control nematodes, but they also control other soil fauna and flora (1) and affect mineralization and nitrification (2). Nematode numbers can be reduced also by nonchemical means, such as by fallowing (3).

This study compares the effects of a residual nematicide, a fumigant, and a nonchemical means of nematode control, on *P. penetrans* numbers, forage yields of alfalfa, and the occurrence of *Fusarium* spp. in plant roots and soil.

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MATERIALS AND METHODS

A field experiment was initiated in 1971 on Charlottetown fine sandy loam at Upton, Prince Edward Island. The site was naturally infested with *P. penetrans* (9,700/kg of soil) and *Fusarium* spp.

Small numbers of *Paratylenchus, Heterodera, Helicotylenchus,* and *Tylenchorhynchus* were also present, although their numbers remained low throughout the experimental period. In May 1972, the number of *Fusarium* propagules was 3100/g of soil. Other soil-borne fungi were present at lower concentrations. There was some winter kill during 1972–73, but damage was uniform over the plot area and recovery of damaged plants was satisfactory. The soil pH was increased to about 6.2 before the experiment. Applications of dolomitic limestone and fertilizer were made each year according to soil analyses and crop recommendations for the area.

In 1971, an area of 24.4 × 24.4-m was subdivided into sixteen 6.1 × 6.1-m plots. Four replicates of four treatments were arranged in a latin-square experimental design. The plots of one treatment were maintained fallow throughout the growing season. The 12 remaining plots were seeded to Lakeland red clover (*Trifolium pratense* L.). All plots were plowed in late October. In the second year of the experiment, the plots of one of the treatments seeded to red clover in the previous year were treated with methyl bromide (bromomethane), the plots of another were treated with phenamiphos [ethyl 4-(methylthio)-m-tolyl isopropylphosphoramidate], and the remainder of the plots seeded to red clover in the previous year were not treated. The methyl bromide was applied on 1 June 1972 at 488 kg/ha under polyethylene buried in the soil to a depth of 20 cm along the edges of the plots. The polyethylene was removed 5 days following fumigation. The phenamiphos (15G) was broadcast by hand using protective clothing on 7 June 1972, at 22.4 kg active ingredient/ha and rototilled to a depth of 10–15 cm. The plots were seeded on 14 June 1972, with Narragansett alfalfa (*M. sativa*) at 13 kg/ha in rows 15 cm apart using a mechanical seeder.

All sampling was from within a 4.6 × 4.6-m area in the centre of each plot. The plants in all plots were cut in August and October of the seeding year (1972) and in late June and August of the succeeding 3 years. Soil samples were collected from each plot before treatment and during September or October of each year after the plots were seeded to alfalfa. Soil samples were mixed thoroughly and screened through a sieve with 2-mm openings. A 50-g subsample of the sieved soil was used for nematode extraction by the modified Baermann pan method (7). Numbers of *P. penetrans* were counted, and infestation levels were calculated as numbers per kg of dry soil. Fungi were isolated from 10-g subsamples of the sieved soil using serial dilutions of the soil suspension added to plates of potato-dextrose agar to which were added 150 ppm streptomycin, 150 ppm neomycin, and 130 ppm pentachloronitrobenzene. After 7–10 days the *Fusarium* colonies were identified and the numbers of propagules per g of dry soil were calculated.

Root samples were collected from each plot during September or October of each year after the plots were seeded to alfalfa. After the roots were washed free of soil, rootlets were trimmed from the main roots and 10 g were incubated under mist for 1 week. *P. penetrans* extracted from the rootlets were counted, and infestation levels were calculated as numbers per g of dry rootlet. A 1-g subsample of the washed rootlets, except in 1975, was washed further under running tap water for 2 h, and 40 sections, 5 mm long, were plated on the same agar medium used for the soil dilutions. Four adjacent 5-mm-long sections beginning at the crown, taken from each of 10 split taproots (a total of 40 sections), were surface-sterilized for 2 min in 1.2% sodium hypochlorite and plated on the agar medium. After 7–10 days the rootlet and taproot sections were examined and the emerging fungi identified. Only data for *Fusarium* spp. are presented.

RESULTS

The numbers of *P. penetrans* in the soil increased from May 1971 to June 1972 in the plots which had grown red clover, and decreased by about 50% in plots fallowed in 1971 (Table 1). At the time of chemical treatment, the plots that had been fallowed

TABLE 1. Effects of cropping and chemical treatments on soil populations of *Pratylenchus penetrans.*

Numbers of *P. penetrans* per kg soil (in 1,000's)[a]								
1971		1972				1973	1974	1975
May	Crop	June	Crop	Chemical treatment	Sept.	Oct.	Sept.	Oct.
10.1 a	red clover	15.9 a	alfalfa	none	20.3 a	30.0 a	18.9 a	11.4 a
9.1 a	fallow	4.5 b	alfalfa	none	0.7 b	20.6 a	26.8 a	19.5 a
10.3 a	red clover	13.8 a	alfalfa	phenamiphos	0.6 b	1.8 b	6.4 b	8.2 a
9.2 a	red clover	14.1 a	alfalfa	methyl bromide	0.0 c	0.0 c	0.0 c	0.1 b

[a]Means in the same column followed by the same letter are not significantly different according to Duncan's multiple-range test (P = 0.05).

had only 30% as many root lesion nematodes as the plots where red clover had been grown. Fallowing in 1971 and phenamiphos and methyl bromide treatment in 1972 resulted in significantly fewer *P. penetrans* in the soil in September 1972, 4 months after chemical treatment and seeding to alfalfa. By October of 1973, and at subsequent samplings, the number of nematodes in the fallowed untreated plots had increased and was not significantly different from that in the untreated plots which had grown red clover. By comparison with the clover-untreated plots, phenamiphos continued to control nematodes through 1973 and 1974, with a gradual loss in control by October of 1975. There was little or no reinfestation of methyl-bromide-treated plots by root lesion nematodes. Numbers of *P. penetrans* in rootlets responded to treatments as did nematode numbers in the soil (Table 2).

Alfalfa forage yield from plots fallowed in 1971 was greater (P = 0.05) than from untreated clover plots only in the year after seeding (Table 3). Yield was greater (P = 0.05) from phenamiphos-treated plots than from untreated clover plots for the second cut in the year of seeding and for season totals in the two following years. Yield from methyl-bromide-treated plots was greater (P = 0.05) than all other treatments for two of the four first cuts, all second cuts, and three of the four season totals.

The number of *Fusarium* propagules in the soil was reduced by methyl bromide fumigation (Table 4), and remained lower than in other treatments for the course of the experiment. Neither fallowing nor phenamiphos treatment affected numbers of *Fusarium* propagules. Only methyl bromide fumigation reduced the percentage of alfalfa rootlets from which *Fusarium* was recovered (Table 4). The percentage of alfalfa taproot sections infected with *Fusarium* spp. tended to be highest after clover and no chemical treatment, though there were no significant differences in this statistic in 1973 and 1975.

Small numbers of propagules of *Rhizoctonia, Phoma, Cylindrocarpon, Trichoderma,* and *Gliocladium* were also recovered from roots. Effects of treatments on these fungi were not studied.

TABLE 2. Effects of successive cropping and chemical soil treatments on numbers of *Pratylenchus penetrans* found in alfalfa roots in four annual samplings.

Treatments	Numbers of *P. penetrans* per g dry rootlet[a]			
	1972	1973	1974	1975
Clover, no chemical, alfalfa	39,600 a	4,610 ab	1,970 a	1,530 a
Fallow, no chemical, alfalfa	2,020 b	5,150 a	3,910 a	2,470 a
Clover, phenamiphos, alfalfa	2,660 b	350 b	270 b	840 a
Clover, methyl bromide, alfalfa	0 c	40 c	20 c	5 b

[a]Sampled in September or October. Means in the same column followed by the same letter are not significantly different according to Duncan's multiple-range test (P = 0.05).

TABLE 3. Effects of successive cropping and chemical soil treatments on forage yields of alfalfa.

Treatments	Forage yield (kg/ha)[a]			
	1972	1973	1974	1975
Clover, no chemical, alfalfa	2,222 b	3,465 c	4,532 b	4,832 b
Fallow, no chemical, alfalfa	2,001 b	4,292 b	4,451 b	4,804 b
Clover, phenamiphos, alfalfa	2,131 b	4,116 b	4,851 a	5,023 b
Clover, methyl bromide, alfalfa	2,886 a	5,832 a	5,136 a	6,364 a

[a]Total from two cuts. Each number is the mean of four replicates. Means in the same column followed by the same letter are not significantly different according to Duncan's multiple-range test (P = 0.05).

DISCUSSION

Phenamiphos treatment resulted in significant nematode control 3 to 4 months following treatment, agreeing with an earlier experience (6). Alfalfa forage yield, however, was not greater at the time of the first cut or for the season total, contrary to the earlier report. Total yield increases were significant for the second and third seasons, coincident with significantly reduced nematode numbers, but not for the fourth season, when nematode numbers were not different from those in untreated plots. The failure of phenamiphos to reduce *Fusarium* infection agrees with earlier observations (6).

Methyl bromide fumigation was very effective in controlling root lesion nematodes and *Fusarium*. Surprisingly, reinfestation of the treated plots by nematodes did not take place within the four seasons included in the present study. Some reinfestation of the soil by *Fusarium* occurred, and *Fusarium* spp. were recovered from taproots and from rootlets, with taproot sections showing progressively more infection with time.

TABLE 4. Effect of successive cropping and chemical soil treatments on *Fusarium* in soil, alfalfa rootlets, and taproots.

Treatments	1972[b]	1972[c]	1973[c]	1974[c]	1975[c]
Number of *Fusarium* propagules per g of soil[a]					
Clover, no chemical, alfalfa	4,900 a	3,500 a	2,300 ab	2,200 a	—
Fallow, no chemical, alfalfa	2,850 a	2,250 ab	3,200 a	1,250 b	—
Clover, phenamiphos, alfalfa	2,200 a	3,400 a	3,100 a	2,250 a	—
Clover, methyl bromide, alfalfa	2,350 a	200 b	300 b	900 b	—
Percentage of alfalfa rootlets infected with *Fusarium*[a]					
Clover, no chemical, alfalfa		100 a	65 a	73 a	—
Fallow, no chemical, alfalfa		98 a	35 b	78 a	—
Clover, phenamiphos, alfalfa		93 a	18 bc	63 a	—
Clover, methyl bromide, alfalfa		45 b	8 c	18 b	—
Percentage of alfalfa taproots infected with *Fusarium*[a]					
Clover, no chemical, alfalfa		38 a	70 a	63 a	65 a
Fallow, no chemical, alfalfa		15 b	53 a	53 b	45 a
Clover, phenamiphos, alfalfa		15 b	38 a	45 bc	45 a
Clover, methyl bromide, alfalfa		13 b	30 a	38 c	47 a

[a]Means in the same column for each source followed by the same letter are not significantly different according to Duncan's multiple-range test (P = 0.05).
[b]Sampled before chemical treatments.
[c]Sampled in September-October.

The treatments in the present study were observed to have different effects on the microorganisms monitored (*Fusarium* and root lesion nematodes), ranging from no effect to very significant reductions. The time required for recovery from these reductions varied with the treatment, from very quick recovery for nematodes in fallowed plots to very slow recovery for both *Fusarium* and nematodes in methyl-bromide-fumigated plots. The return of nematode numbers to levels approximating those in check plots was a function of the levels to which they were reduced initially by the treatments. Fallowing reduced the numbers least and allowed more rapid return, while methyl bromide reduced the numbers to below-detectable levels, and allowed only very slow return. Initial control of nematodes by phenamiphos was comparable to that from fallowing. However, nematode numbers returned much more slowly in phenamiphos-treated plots, presumably because of the residual nature of the nematicide.

Fusarium infections of rootlets and taproots were influenced by numbers of nematodes infesting roots. The fewer *Fusarium* infections in rootlets and taproots from methyl-bromide-fumigated plots is not surprising. The reduced infections in phenamiphos-treated, and at some samplings in fallowed plots, can be related only to the reduced numbers of nematodes infesting the roots. This is in agreement with an earlier observation (6) where *Fusarium* infections were observed to be less in phenamiphos-treated plots coincident with reduced nematode numbers.

While no differences were observed in the persistence of alfalfa plants among the treatments included in this study, increased infections by *Fusarium* were expected to contribute to increased root rot (4) and ultimate deterioration of the root system such that individual plants would become unproductive and/or die prematurely, either as a direct result of the root rotting or indirectly because of additional stress such as severe winter conditions.

The present results confirm that the yield potential of alfalfa is being reduced significantly, with *P. penetrans* infestation, and probably *Fusarium* infections contributing to the reduction. Understanding of the respective contributions of the two organisms requires study.

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