Effects of Soil pH on Reproduction of Pratylenchus penetrans and Forage Yield of Alfalfa[1]

C. B. WILLIS[2]

Abstract: 'Vernal' alfalfa was grown for 30 weeks in nematode-free soil and in soil infested with *Pratylenchus penetrans*. Charlottetown fine sandy loam soil was used at its pH of 4.4 and at adjusted reactions of 5.2, 6.4 and 7.3. Nematode reproduction was significantly greater at pH 5.2 and 6.4 and was not related to alfalfa root production over the full pH range studied. A significant nematode infestation × soil pH interaction on forage yield was recorded. Nematode infestation significantly decreased forage yields at pH 5.2 and 6.4 but not at pH 4.4 and 7.3. *Key Words:* root lesion nematode, interaction, *Medicago sativa*, soil reaction.

Growth of alfalfa under greenhouse conditions is affected adversely by the endoparasitic nematode, *Pratylenchus penetrans* (Cobb) Filip. and Schur. Stek. (4). The influence of soil reaction (pH) on *Pratylenchus* spp. has not been studied extensively, and little information is available on the effects of interactions between soil pH and nematode infestation on plant growth. Coarse root of tobacco, caused by *Pratylenchus* spp., increased with increasing soil alkalinity within the range of pH 5.2 to 6.2 (6); however, nematode population levels associated with disease ratings were not given. Greater numbers of *P. penetrans* were recovered from roots of vetch grown in soil at pH 5.8 than at 4.8 or 7.5 (8). Populations of *P. alleni* Ferris colonizing soybean roots at pH 6.0 were significantly greater than at pH 4.0 or 8.0 (3). Greater numbers of *P. crenatus* Loof and *Tylenchorhynchus dubius* Bütschli were found associated with plants cultured in acidic soils than in alkaline soils (1, 2). *Heterodera avenae* Wr., *Tylenchorhynchus maximus* Allen, *Criconemoides xenoplax* Raski, and *Tylenchulus semipenetrans* Cobb increased in numbers as soil alkalinity increased (5, 10, 11, 13). Egg hatch of *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub.) Chitwood was optimum at pH 6.4-7.0 (7, 14).

The objectives of this study were to determine (i) the effect of soil pH on the reproduction of *P. penetrans* on alfalfa; and (ii) the effects of soil pH and nematode infestation on forage yield of alfalfa under greenhouse conditions.

MATERIALS AND METHODS

Charlottetown fine sandy loam, pH 4.4, was fumigated with methyl bromide at the rate of 454 g/500 kg soil to eliminate nematodes. A commercial fertilizer, 5-20-20-2B, was mixed with the soil at the rate of 0.4 g/kg. After the soil was thoroughly mixed, a 3:2 mixture of

Received for publication 17 January 1972.

[1]Contribution No. 250, Research Station, Canada Department of Agriculture, Box 1210, Charlottetown, Prince Edward Island, Canada. The technical assistance of A. W. MacMicken is gratefully acknowledged.

[2]Plant Pathologist, Canada Department of Agriculture, Box 1210, Charlottetown, Prince Edward Island, Canada.

powdered $CaCO_3$ and $MgCO_3$ was added to separate portions to adjust the pH to 5.2, 6.4 and 7.3. The amounts of the mixture added and the resulting pH values after a 4-week adjustment period are presented in Table 1. The pH of the soil was determined in a 1:1 soil-to-water mixture.

The soil was placed in 5-liter polyethylene pots, and half the pots at each pH level were infested with nematodes by a thorough mixing of a water suspension of *P. penetrans* with the soil in the upper half of each pot. Approximately 7800 nematodes were added to each pot. The nematode inoculum was obtained from soil by modified Baermann pan extraction (12) from greenhouse stock cultures of *P. penetrans* maintained on birdsfoot trefoil (*Lotus corniculatus* L.). Fifteen 2-day-old germinated seeds of alfalfa (*Medicago sativa* L. 'Vernal') were planted in each pot and thinned to five plants/pot after 3 weeks. A commercial alfalfa strain of *Rhizobium* was added to all pots at seeding.

A split, split-plot experimental design with four replications was used. Main plot treatments were infested with *P. penetrans* and noninfested controls. Subplots were soil pH levels, and sub-subplots were total plant harvests (forage and roots) when forage cuts 1, 3 and 6 were taken at 9, 18 and 30 weeks from seeding. Forage was also cut, but roots were not harvested, at 14, 21 and 26 weeks after seeding (cuts 2, 4 and 5).

Pots were maintained in a greenhouse at 22 ± 2 C during the day and 14 ± 2 C at night. Supplementary light (approximately 11,000 lux) was provided to give a 14-hr daily photoperiod. All pots were watered initially by weight to field capacity of the soil, and watered again only when the soil moisture content of any pot reached 70% of field capacity. Plants were cut back to a height of 4 cm when a minimum of one-quarter of the plants had reached the flowering stage. Over a 210-day period, forage was harvested six times; yields were based on oven-dry (80 C for 24 hr) weights of the forage.

The soil pH in each pot was determined at the time of seeding and at the time of each total plant harvest. Alfalfa roots were carefully removed from the soil and cut into small pieces, and a maximum of 10 g was placed on Baermann funnels under intermittent mist (3 min in 10) (9). Nematodes were counted after 1 week of incubation, and the numbers per gram dry weight of rootlet tissue were calculated. The number of nematodes in the soil was determined by extraction of nematodes from 50 g of soil using the modified Baermann pan method.

Data on numbers of nematodes were subjected to log transformations before statistical analysis. All data were subjected to analysis of variance, and Duncan's multiple range test for significance was applied to treatment means.

TABLE 1. Soil pH after adding $CaCO_3$ + $MgCO_3$ and growing alfalfa in soil infested with *Pratylenchus penetrans* and in noninfested soil.

$CaCo_3$ + $MgCO_3$ added[a] (g/kg)	Treatment[b]	Soil pH at weeks after seeding alfalfa 0[c]	9	18	30
0	C	4.4	4.7	4.6	4.6
0	N	4.4	4.7	4.6	4.5
1.3	C	5.2	5.1	5.2	5.5
1.3	N	5.2	5.1	5.4	5.5
3.5	C	6.4	6.1	6.2	6.3
3.5	N	6.4	6.1	6.2	6.3
8.4	C	7.3	7.4	7.4	7.5
8.4	N	7.3	7.4	7.5	7.6

[a] A 3:2 mixture of $CaCO_3$ and $MgCO_3$.

[b] Treatments include a noninfested control (C) and Pratylenchus penetrans infested (N).

[c] Four weeks after addition of $CaCO_3$ + $MgCO_3$.

RESULTS

Slight changes occurred in the soil pH from the time of seeding to the time of complete plant harvests at 9, 18 and 30 weeks (Table 1). No change greater than 0.4 of a pH unit was recorded, and at final harvest, soil pH had increased slightly except when the pH at seeding was 6.4.

Alfalfa forage yield was not significantly affected by nematode infestation until cuts 5 and 6, taken 26 and 30 weeks after seeding, respectively (Table 2). Total cumulative forage yield for the 30-week experimental period was also significantly affected by nematode infestation. Soil pH had a significant effect on forage yield throughout the experiment. The interaction of nematode infestation X soil pH on forage yield was highly significant at cuts 5 and 6 and for the total.

When the soil pH at seeding was either 4.4 or 7.3, nematode infestation had no significant

TABLE 2. Analysis of variance of forage yield by alfalfa.

Variable	Mean squares						
	Cut 1 (9)[a]	Cut 2 (14)	Cut 3 (18)	Cut 4 (21)	Cut 5 (26)	Cut 6 (30)	Total
Replications	0.98	2.29*[b]	2.20*	1.10	0.69*	1.02	3.57
Nematode infestation (N)	0.26	0.01	1.13	2.10	12.63**	27.38**	102.60*
Error A	0.34	0.46	0.17	0.78	0.05	0.16	7.49
Soil pH	49.05**[c]	50.64**	20.49**	36.63**	43.20**	57.07**	783.05**
N X Soil pH	0.26	0.05	0.11	1.05	2.37**	5.86**	28.97**
Error B	0.34	0.55	0.33	0.40	0.46	0.39	5.10
(Number of replications)	(12)	(8)	(4)	(4)	(4)	(4)	(4)

[a]Numbers in parentheses are weeks after seeding. [b] *.05>P>.01. [c]**P<.01.

effect on alfalfa forage yield; however, nematode infestation significantly reduced yields at soil pH 5.2 and 6.4 (Table 3). Forage yield at a soil pH of 4.4 was significantly less than at higher pH values. Yield at pH 5.2 was significantly lower than at 6.4 and 7.3, except that total yield from noninfested soil at pH 5.2 and 7.3 did not differ. Maximum yield from nematode-infested plants was obtained at a higher soil pH (7.3) than from noninfested plants (pH 6.4). Nematode infestation decreased forage yield most at soil pH 5.2 and least at soil pH 7.3 (Fig. 1).

Alfalfa root production was not affected significantly by nematode infestation but was by soil pH (Fig. 2). Root production was poor at pH 4.4, and generally paralleled forage yield.

Significantly more nematodes were recovered from rootlets growing in soil at pH 4.4 than at higher levels after 9 weeks (Fig. 3); however, after 18 and 30 weeks, significantly greater numbers were recovered at pH 5.2 and 6.4 than at either pH 4.4 or 7.3. Nematode

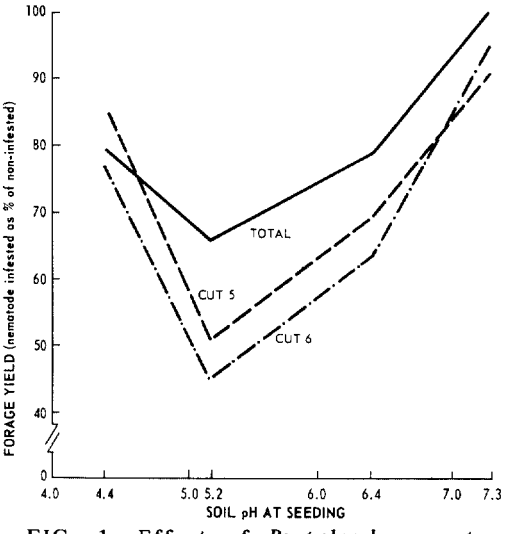

FIG. 1. Effect of *Pratylenchus penetrans* infestation and soil pH on alfalfa forage yield.

TABLE 3. Interaction effects of *Pratylenchus penetrans* and soil pH on forage yield of alfalfa.

Soil pH at seeding	Forage yield (g/pot)					
	Cut 5[a]		Cut 6		Total	
	C[b]	N[c]	C	N	C	N
4.4	0.8 e	0.7 e	1.8 e	1.4 e	4.0 d	3.2 d
5.2	4.9 bc	2.5 d	6.5 b	2.9 d	22.3 b	14.7 c
6.4	6.1 a	4.2 c	8.0 a	5.0 c	28.0 a	22.0 b
7.3	6.4 a	5.8 ab	8.0 a	7.6 a	24.1 b	24.1 b

[a]Means within a harvest followed by the same letter are not significantly different at P = .05.

[b]C = noninfested soil.

[c]N = soil infested with P. penetrans.

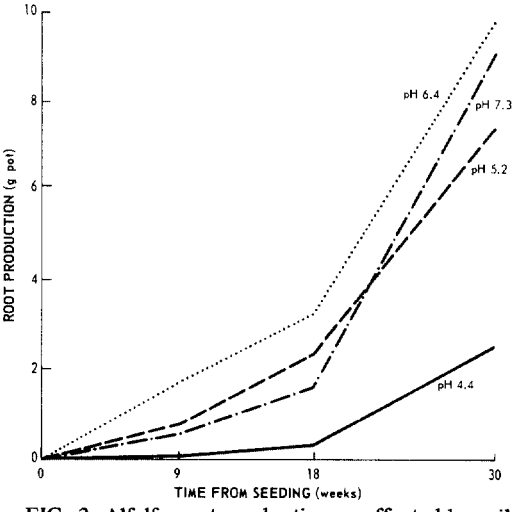

FIG. 2. Alfalfa root production as affected by soil pH.

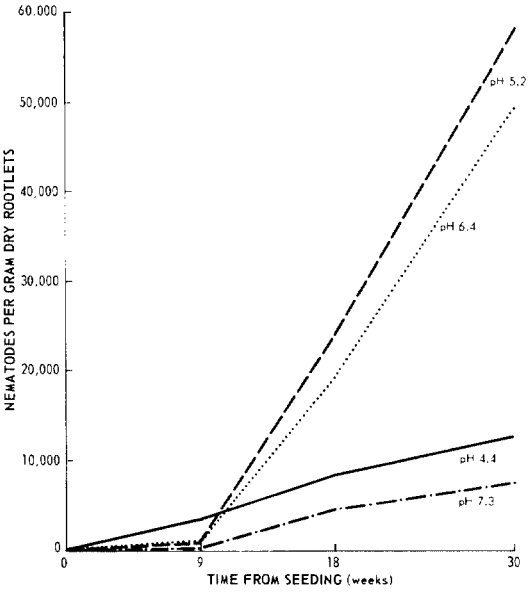

FIG. 3. Effect of soil pH on numbers of *Pratylenchus penetrans* in alfalfa roots.

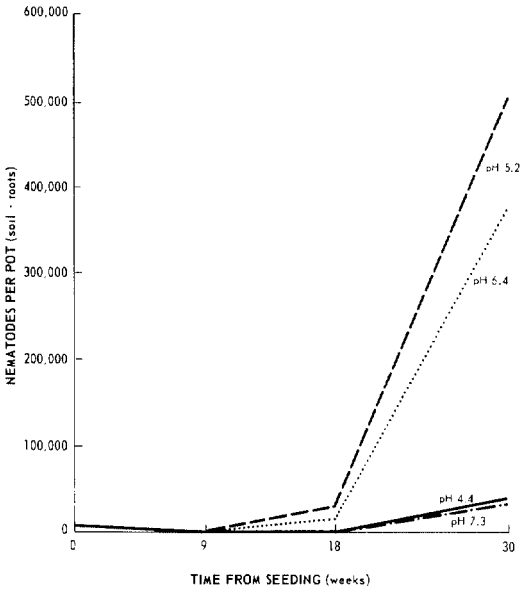

FIG. 4. Effect of soil pH on *Pratylenchus penetrans* reproduction on alfalfa.

recovery from rootlets was not correlated with root production over the full pH range of 4.4 to 7.3. Nematode reproduction was much greater at pH 5.2 and 6.4 than at either 4.4 or 7.3 (Fig. 4). Greatest nematode reproduction, which occurred at pH 5.2, resulted in the greatest forage yield decrease (Fig. 1).

DISCUSSION

The data presented in this paper indicate that the effect of soil reaction on the reproduction of *P. penetrans* is not via the host plant over the total pH range considered, but could be over the lower part of the pH range. Root production and nematode reproduction were poor at pH 4.4, but at pH 7.3, root production was as good as at pH 5.2 and 6.4, whereas nematode reproduction was significantly less at the highest pH. Optimum nematode reproduction recorded at soil pH 5.2 and 6.4 in this study compares favorably with the optimum pH for egg hatch of *M. incognita* and *M. javanica* (7, 14). Our data, showing that alfalfa roots growing in soil at pH 5.2 and 6.4 supported more *P. penetrans* per gram than at lower or higher pH levels, agree with results of Morgan and MacLean (8) for *P. penetrans* in vetch roots, and of Burns (3) for *P. alleni* in soybean roots. Reproduction of *P. penetrans* on alfalfa, like that of *T. semipenetrans* on sweet orange (13), was poor at pH 4.4 and good at 5.2 and 6.4; but at pH 7.3, unlike *T. semipenetrans*, *P. penetrans* did not reproduce well.

The relatively low nematode population level (780/454 g soil) used in this study resulted in a slow population increase and a level of root infestation which caused no detrimental effects on root production.

Forage yield was not significantly affected by nematode infestation until 26 weeks after seeding, and then only at the intermediate pH 5.2 and 6.4, where nematode reproduction was much greater by 18 weeks after seeding. Forage yield reduction from nematode infestation was not as great at pH 6.4 (near the recommended soil pH for successful alfalfa production in Eastern Canada) as at pH 5.2 (Fig. 1), but was considerably greater than at pH 7.3. Total forage yield from alfalfa was optimum at pH 6.4, and yield was depressed at pH 7.3 in nematode-free but not in nematode-infested soil. The observed interaction between *P. penetrans* and soil pH on alfalfa forage production, therefore, is significant in determining optimum growing conditions for alfalfa.

LITERATURE CITED

1. BRZESKI, M. W. 1969. Nematodes associated with cabbage in Poland. II. The effect of soil factors

on the frequency of nematode occurrence. Ekol. Pol. Ser. A. 17:205-226.

2. BRZESKI, M. W. and A. DOWE. 1969. Effect of pH on *Tylenchorhynchus dubius* (Nematoda, Tylenchidae). Nematologica 15:403-407.

3. BURNS, N. C. 1971. Soil pH effects on nematode populations associated with soybeans. J. Nematol. 3:238-245.

4. CHAPMAN, R. A. 1958. The effect of root-lesion nematodes on the growth of red clover and alfalfa under greenhouse conditions. Phytopathology 48:525-530.

5. DUGGAN, J. J. 1963. Relationship between intensity of cereal root eelworm (*Heterodera avenae* Wollenweber 1924) infestation and pH value of soil. Irish J. Agr. Res. 2:105-110.

6. KINCAID, R. R. and N. GAMMON, JR. 1957. Effect of soil pH on the incidence of three soil-borne diseases of tobacco. Plant Dis. Rep. 41:177-179.

7. LOEWENBERG, J. R., T. SULLIVAN and M. L. SCHUSTER. 1960. The effect of pH and minerals on the hatching and survival of *Meloidogyne incognita incognita* larvae. Phytopathology 50:215-217.

8. MORGAN, G. T. and A. A. MAC LEAN. 1968. Influence of soil on an introduced population of *Pratylenchus penetrans*. Nematologica 14:311-312.

9. OOSTENBRINK, M. 1960. Estimating nematode populations by some selected methods, p. 85-102. *In* J. N. Sasser and W. R. Jenkins [eds.]. Nematology: Fundamentals and Recent Advances, Univ. North Carolina Press, Chapel Hill. 480 p.

10. SCHMITT, D. 1969. Population patterns of some stylet-bearing nematodes in a native Iowa prairie. J. Nematol. 1:304 (Abstr.).

11. SESHADRI, A. R. 1964. Investigations on the biology and the life cycle of *Criconemoides xenoplax* Raski, 1952 (Nematoda:Criconematidae). Nematologica 10:540-562.

12. TOWNSHEND, J. L. 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cotton-wool filter extraction method. Nematologica 9:106-110.

13. VAN GUNDY, S. D. and J. P. Martin. 1961. Influence of *Tylenchulus semipenetrans* on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. Phytopathology 51:146-151.

14. WALLACE, H. R. 1966. Factors influencing the infectivity of plant parasitic nematodes. Roy. Soc. B. Biol. Sci. Proc. 164:592-614.