Influence of Paratylenchus projectus on alfalfa sickness in Alberta[1]

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Despite high populations of *Paratylenchus* projectus Jenkins (pin nematodes), healthy samples of grey wooded Luvisolic soil from central Alberta were significantly more productive of Grimm alfalfa growth than were samples of virgin or alfalfa-sick soils tested. The difference was due to higher fertility and pH. Grimm alfalfa consistently reduced populations of pin nematodes that had been increased by preplanting with Lotus corniculatus L. (bird's-foot trefoil). Comparably high populations of pin nematodes elsewhere in Alberta did not produce alfalfa sickness.

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Malgre la presence de fortes populations de *Paratylenchus* projectus Jenkins (nematode acumine), on a constaté que des echantillons de sols Luvisols gris boisés du centre de l'Alberta produisaient davantage de luzerne Grimm que des echantillons de sols vierges ou montrant des signes de "fatigue" de la luzerne. Cette difference se justifie par le meilleur etat de fertilité et le pH plus élevé de ces sols. La culture de luzerne Grimm a regulierement reduit les populations de nematodes qu'avait accrues une plantation anterieure de Lotus corniculatus L. (Lotier cornicule). Ailleurs en Alberta, des populations relativement fortes de nematodes n'ont pas provoque de symptômes de "fatigue" de la luzerne.

Introduction

Alfalfa sickness, reported by Webster et al. in 1967 (13), occurred in alfalfa (*Medicago sativa* L.) growing in Dark Grey Luvisolic soils of central Alberta. Affected plants were short, spindly, and yellowish green and had little or no nodulation and poorly developed root systems. In affected stands, growth was retarded but healthy plants were frequently found in irregular patches throughout the fields.

The Dark Grey Luvisolic soils were characteristically of low pH (5.8-6.0), low in nitrogen (N), phosphorus (P), sulfur (S), and humus (7, 9). Neither fertility level nor moisture content were critical factors in disease development in field tests (12, 13). None of a wide range of alfalfa varieties tested was resistant to alfalfa sickness (3). Mild heat (51°C) frequently eliminated the disease (3) and indicated that a toxin might be an incitant.

A pin nematode, *Paratylenchus projectus* Jenkins (5), was found in alfalfa-sick soils by the late W. R. Orchard (14) and identified by L. Y. Wu of Biosystematics Research Institute, Agriculture Canada. Populations were generally greater in the rhizospheres of 'sick' plants (11).

Coursen et al. (1) listed 89 hosts of *P. projectus* in 1958. These included alfalfa, red clover, oats, bromegrass, and timothy -- all grown in central Alberta areas of alfalfa sickness. The host range of the pin nematode and its association with alfalfa sickness in the Grey Luvisolic soils of central Alberta (4, 9, 11, 14) suggested that the nematode might be closely involved in this serious plant disease.

In this study of alfalfa sickness, the effect of initial pin nematode population, soil source, pH, and *Rhizobium* was examined.

Materials and Methods

Soils were selected from the University of Alberta soil science plots at Breton, Alberta, where the soils are luvisolic and alfalfa sickness has been reported (13). The plots sampled were virgin (BH), healthy (BD7), and sick (BD3). The "Virgin" soil was low in N, P, and S (7), unfertilized, and pH 5.2 and contained 752 pin nematodes/kg (6, 8). "Healthy" and "Sick" soils came from adjacent plots of 2-year-old Grimm alfalfa. The "Healthy" plot was on a complete fertilizer (N, P, and K) program with additional lime (L) and sulfur (S), was pH 5.8, and contained 41,300 pin nematodes/kg. The "Sick" plot was on a complete fertilizer program (excluding L), was pH 5.4, and contained 74,400 pin nematodes/kg.

Half of the soil taken from each plot was planted to *Lotus corniculatus* L. cv. Leo (bird's-foot trefoil) for 3 months to increase the populations of pin nematodes. The counts per kilogram of soil were: virgin - 768, healthy - 6,280, sick - 145,880. All plant debris was removed before seeding with alfalfa was begun. The remaining soil was stored at ambient room temperature without watering.

Elements or factors considered in this study of alfalfa sickness were type of soil, effect of pH, effect of initial pin nematode population, effect of preplanting with bird's-foot trefoil, and influence of inoculating the soils

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Table 1. Means* and standard errors for total yield of Grimm alfalfa top growth, plant weight, and final Paratylenchus projectus population**:* for the three soil sources and influence of preplanting with bird's-foot trefoil

	Variables and bird's-foot trefoil levels					
	Top growth (g)		Plant root and crown weight (g)		No. of *P. projectus* per kg of soil	
Soil source	Not preplanted	Preplanted	Not preplanted	Preplanted	Not preplanted	Preplanted
Virgin (BH)	11.82a	13.33a	13.44bc	14.76ab	280c (740)	770b (1,610)
Healthy (BD7)	13.21a	13.44a	16.94a	14.80ab	1,450b (1,970)	85,680a (92,620)
Sick (BD3)	12.44a	10.06b	11.73cd	11.01d	1,230b (2.090)	227,720a (264,450)
Standard errors (71 df)	0.397		0.570			0.127

*For each variable, means followed by the same letter do not differ significantly ($P > 0.05$) using Tukey's test (2).
**For numbers of *P. projectus,* geometric means are given and the standard error is in $\log_{10}$ units. Arithmetic means are in brackets.

Table 2. Means* and standard errors for total yield of Grimm alfalfa top growth, plant weight, and final Paratylenchus projectus population**:* for three soil sources and two lime treatments

	Variables and lime treatments					
	Top growth (g)		Plant root and crown weight (g)		No. of *P. projectus* per kg of soil	
Soil sources	None	pH 6.5	None	pH 6.5	None	pH 6.5
Virgin (BH)	10.01d	15.15a	12.63b	15.57a	430b (1,290)	505b (1,060)
Healthy (BD7)	12.17c	14.49ab	15.49a	16.25a	10,510a (53,100)	11,820a (41,490)
Sick (BD3)	9.46d	13.03bc	10.98b	11.76b	18,480a (123,000)	15,170a (143,540)
Standard error (71 df)			0.397	0.570		0.127

*For each variable, means followed by the same letter do not differ significantly ($P > 0.05$) using Tukey's test (2).
**For numbers of P. *projectus,* geometric means are given and the standard error is in $\log_{10}$ units. Arithmetic means are in brackets

with the recommended strain of *Rhizobium meliloti* Dangar.

Each combination of soil and nematode population level was placed in sixteen 15-cm clay pots and seeded with five Grimm alfalfa seeds per pot. Treatments were: (1) control, (2) lime to raise soil pH to 6.5, (3) inoculate the soil with *Rhizobium*, (4) lime to raise soil pH to 6.5 and inoculate with *Rhizobium*.

All combinations of the soil types and treatments formed a 3 (soils) X 2 (nematode levels) X 2 (lime) X 2 (*Rhizobium* inoculum) factorial experiment that was set out in four randomized blocks on a greenhouse bench. Watering was controlled to maintain the soil at 25% of field capacity. Uniform light was supplied by fluorescent light banks regulated to give illumination for 16 h/day at 21,530 lux. The temperature was maintained at 20°C ± 1".

Top growth from each test plot was collected at six regularly-spaced intervals from 23 January to 22 August 1973. It was bagged, dried at 75°C for 48 h, and then weighed. On completion of the test, data were compiled on top growth, total weight including roots and crowns, soil pH (Fisher Accumet), populations of pin nematodes, and extent of *Rhizobium*-induced nodule growth. Analyses of variance (2) were carried out on the above data. A logarithmic transformation was applied to

Table 3. Means':' and standard errors of ***Rhizobium meliloti*** levels on total yield of top growth, plant weight, ***Paratylenchus projectus*** populations**, and pH

	Variables			
R. meliloti level	Top growth (g)	Plant weight (g)	No. of P. ***projectus***/kg	Soil pH
Control	12.38a	13.54a	4,765a (65,290)	6.53a
Inoculated	12.39a	14.01a	4,110a (55,870)	6.53a
Standard error (71 df)	0.229	0.329	0.073	0.012

*For each variable, means followed by the same letter do not differ significantly (P 7 0.05) using Tukey's test (2).
"'For numbers of ***P. projectus,*** geometric means are given and the standard error is in $\log_{10}$ units. Arithmetic means are in brackets.

Table 4. Soil pH means'' and standard error for three soils, two nematode population levels, and two lime treatments

		Lime treatment	
Soil	Pin nematode populations	None	To pH 6.5
Virgin (BH)	Low	5.83i	6.94abc
	High	5.96hi	6.91bc
Healthy (BD7)	Low	6.25f	7.08a
	High	6.62e	6.72de
Sick (BD3)	Low	6.12fg	7.04ab
	High	6.08gh	6.82ce
Standard error (71 df)		0.029	

Means followed by the same letter do not differ significantly (P 7 0 05) using Tukey's test (2)

the pin nematode counts to make the treatment means and variance independent.

Results and discussion

Although the total alfalfa yields of top growth and weights of plant roots and crowns were not significantly affected by preplanting in virgin or healthy soils, top growth yields were lower ($P < 0.05$) in pots of sick soil that had been preplanted with bird's-foot trefoil (Table 1). Populations of pin nematodes after alfalfa growth were higher ($P < 0.05$) in soils that had been preplanted to bird's-foot trefoil, especially the sick soil, which had low pH and no previous history of liming.

The contrasting yields of top growth in healthy and sick soils showed that the former owed its high productivity to a residual influence of NPKSL fertilizers that resulted in pH 5.8 and production of good yield despite high populations of pin nematodes in preplanted test portions of this soil. When compared to virgin soil, weights of plant roots and crowns were reduced in sick soil but not in healthy, despite significantly greater populations of pin nematodes (Tables 1 and 2).

Lime treatment increased total yield and plant weight (Table 2). The largest increase (51%) in total yield due to lime treatment occurred in virgin soil, which had no previous NPKS or L fertilizers and had the lowest pH. In contrast, the healthy soil, which had had prolonged NPKSL treatment, showed the least increase (19%) and the sick soil, which had received NPKS, gave an intermediate increase (37%).

Lime treatment had no significant effect on the numbers of pin nematodes (Table 2) but its effect on pH was greater in virgin than in healthy soil (Table 4) probably because of previous use of lime in the healthy plot.

Inoculation of alfalfa seed with ***R. meliloti*** neither promoted nodulation nor significantly increased yield of alfalfa grown in the test soils (Table 3). This indicated a

need for a viable strain of the bacterium better able to cope with the Grey Luvisolic soil environment.

High populations of pin nematodes were found in the rhizospheres of healthy alfalfa in the Leduc district (11) and, in southern Alberta, pin nematodes were detected in 52% of the irrigated stands examined. Also, in a survey of irrigated alfalfa-grass pasture soils at the Lethbridge Research Station extending from 1973 through 1975, numerous pin nematodes were present in 71% of the samples examined.

Alfalfa consistently reduced pin nematode populations in this study, a result confirmed by Townshend and Potter (10), who found bird's-foot trefoil, timothy, and clovers were good hosts. These hosts are grown in and out of the areas of alfalfa sickness in Alberta and would, if the pin nematode was the principal cause of the disease, contribute greatly to disease development and spread through increase in nematode population.

The reduced yield of alfalfa from preplanted sick soil ($P < 0.05$) compared to preplanted healthy soil ($P < 0.05$) showed the value of NPKSL fertilizers (Table 1). It is evident that such management together with the poor host capabilities of the alfalfa minimized the role of the pin namatode in alfalfa sickness in Alberta.

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