Seed preinoculation and soil liming for growth of forage legumes on acidic clay soils

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SUMMARY

Forages produced in the north central interior of British Columbia are low in percentage crude protein (CP). Growing lucerne (*Medicago sativa* L.) should increase forage percentage CP, but many of the soils are considered too acidic for this species. The objective of three field experiments, conducted at the Prince George experimental farm, was to evaluate management practices that might accommodate the growth of lucerne on acid soils without the expense of liming. Experiment 1 compared three legume species (lucerne, alsike clover (*Trifolium hybridum* L.) and red clover (*T. pratense* L.)), preinoculated lime-coated seed (GNR™, Grow Tec Ltd, Nisku, Alberta), and soil liming on root nodulation and forage dry matter (DM) yield. Experiment 2 compared lucerne genetic lines, seed preinoculation and soil liming on root nodulation and forage DM yield and percentage CP. Experiment 3 compared lucerne rhizobia genetic strains, seed preinoculation and soil liming on root nodulation, forage DM yield and percentage CP.

For effective nodulation, lucerne required seed preinoculation, whereas alsike clover and red clover did not. Lucerne persisted longer than alsike or red clover. In Expts 2 and 3, the combination of lime and preinoculation increased lucerne DM yield by 136% and %CP from 9·2 to 15·4. The addition of lime alone increased lucerne DM yield by an average of 130% and %CP from 9·2 to 16·3. With preinoculation alone, lucerne DM yield increased by 100% and %CP increased from 9·2 to 12·7. Although using preinoculated lime-coated seed alone is not as effective as lime alone, coated seed may be the preferred method, based on ease of application and cost. Neither the six lucerne lines nor the three rhizobial strains evaluated resulted in improved root nodulation under acidic conditions.

INTRODUCTION

Predominantly non-legume forages, low in percentage crude protein (%CP), are produced in central interior British Columbia, and producers would like to increase %CP by growing a persistent legume such as lucerne (*Medicago sativa* L.). About 53% of the agricultural soils in this region of British Columbia have a pH < 6·0 (Broersma 1987). Liming soils in this region to increase the growth of lucerne is not economic at present as there is no local source of agricultural lime (J. Tingle, personal communication).

Rice *et al.* (1977) studied soils from northern British Columbia and Alberta within a pH range of 4·5–7·2. Below pH 6·0, *Rhizobium meliloti* numbers in the soil, nodulation rating and lucerne dry matter (DM) yield all decreased with decreasing pH. In contrast, nodulation and growth of red clover was not affected within the pH range 4·5–7·2. However, the number of *R. leguminosarum* var. *trifolii*, that nodulated red clover, decreased when pH was < 4·9.

Nodulation of clover when sown on a field scale into a moderately acid soil (pH 5·5) was numerically higher for plants from lime-coated seed than from bare seed (Hely 1965). Lime-coated preinoculated clover seed is convenient to use because it can be stored for several weeks and rhizobia viability remains high (Stout *et al.* 1993). Rice & Olsen (1983) inferred that the use of coated seed would provide an effective method of inoculating lucerne sown in soil of pH 5·8–5·9.

The objective of this study was to evaluate management practices that might facilitate lucerne production on moderately acid soils without liming. We evaluated the use of lime-coated preinoculated seed to provide rhizobia to lucerne and clovers, the use of different lines of lucerne for adaptability to acid soil, and the use of different strains of rhizobia for

Table 1. *Summary of treatments for the three split-plot experiments. Main plots were lime or no lime*

Factor	Expt 1	Expt 2	Expt 3
Forage species Cultivar/line	Lucerne (Peace) Red clover (Canada common No. 1) Alsike clover (Canada common No. 1)	Lucerne (Peace) (Sure) (Oneida VR) (Ambassador) (Breeder line 1) (Breeder line 2)	Lucerne (Peace) (Sure) (Oneida VR)
Rhizobial strain	NRG185	NRG185	NRG185 GT004 GT005
Seed treatment	Bare Preinoculated	Bare Preinoculated	Bare Preinoculated
Lime	0 t ha^{-1} 2·3 t ha^{-1}	0 t ha^{-1} 5 t ha^{-1}	0 t ha^{-1} 5 t ha^{-1}
No. of blocks	4	3	3

adaptability to acid soil. Limed control plots were included in all three field experiments.

MATERIALS AND METHODS

Site and soil

Three field experiments were conducted at the Prince George British Columbia experimental farm (53° 54′ N, 122° 43′ W, 670 m above sea level) from 1989 to 1994. The soil is a clayey Gleyed Gray Luvisol (Canada Soil Survey Committee 1978) (Aquic Cryoboralf; Soil Survey Staff 1975) of the Pineview soil series, with a surface horizon pH of 5·1(H_2O) (Farstad & Laird 1954). Such Luvisolic soils developed from glacial till and lacustrine deposits, low in organic matter, moderately to strongly acidic and with a restrictive B horizon due to clay accumulation, are representative of the agricultural soils in the region (Lavkulich 1980).

Experimental design

In all three experiments, plots consisted of eight 6·1 m rows at 30 cm spacing. Sowing rates were 10, 8, and 6 kg ha^{-1} for lucerne, red clover (*Trifolium pratense* L.) and alsike clover (*Trifolium hybridum* L.), respectively. Seed treatments were bare uninoculated seed or lime-coated (GNR™, Grow Tec Ltd, Nisku, Alberta) preinoculated seed. Samples of the same seed lot were used for bare and lime-coated seed. The coating process applied peat-based inoculum and a lime coating. Coating increased seed weight by an average of 48% for lucerne and red clover, and by 83% for alsike clover. Coated seed was stored in the dark at 5 °C until sowing. Sowing rates for coated seed were corrected to give the same number of seeds ha^{-1} as for bare seed. A split-plot design was used; one main plot within a replicate had no lime and the other received 36% agricultural grade lime applied prior to sowing. The species, lucerne lines or rhizobia strains and inoculation treatments were randomized within the two main plots and treatments were replicated over three or four blocks.

Experiment 1 compared nodulation and yield of three legume species (lucerne, alsike clover and red clover) with and without the application of lime and with or without seed preinoculation (Table 1). Before sowing this experiment, 200 kg ha^{-1} of 13:16:10 ($N:P_2O_5:K_2O$) was worked into the soil during plot preparation. Plots were sown on 9 June 1989. Seedling counts and nodulation evaluations were obtained on 9 August 1989. Dry matter yield was determined on 20 July 1990, 11 July and 25 September 1991 and on 29 September 1992.

Experiment 2 compared nodulation and yield of four cultivars and two breeder-selected lines of lucerne applied with or without lime and with or without seed preinoculation (Table 1). Because of limited seed, the breeder lines were preinoculated and sown on the no lime treatment only. Plots were sown on 16 June 1992. Nodulation evaluations were performed on 7 October 1992 and on 8 June 1993. This experiment was harvested on 8 July and 7 September 1993.

Experiment 3 compared nodulation and yield of bare or preinoculated seed with one of three rhizobia strains, three lucerne cultivars and lime (Table 1). Rhizobia strain NRG185 is the Agriculture and Agri-Food Canada strain available in several commercial inoculants sold in western Canada. Experimental strains, GT004 and GT005, were obtained by Grow Tec Ltd. In Expts 2 and 3, gamma-irradiated sterile peat (Yellowstone Valley Chemicals, Montana) was inoculated with the various strains of rhizobia by Grow Tec Ltd on 8 October 1991. Seed was preinoculated and coated on 9 June 1992. Plate counts to determine colony forming units (CFU) per seed (Olsen & Rice 1989) were conducted by Grow Tec Ltd on 29 June 1992 for the cultivar Peace only. The resulting values were 2850 CFU for seed inoculated with NRG185; 21200 CFU for seed inoculated with GT004; and 35000 CFU for seed inoculated with GT005. Using the grow-out technique (Agriculture Canada 1991) and Peace seed, the Grow Tec laboratory estimated a most probable number (MPN) of > 35500 for seed inoculated with all three rhizobia strains. Experiment 3 was sown on 17 June 1992. Nodulation evaluations were made on 8 October 1992 and on 9 June 1993. This experiment was harvested on 9 July and 8 September 1993.

Plant measurements

Evaluation of seedling establishment and root nodulation

In Expt 1, seedling counts were made by digging plants from within a 30 cm section of a row from each plot. Percentage establishment was calculated from the number of seedlings counted and the total number of seeds sown.

To determine percentage of plants nodulated in Expt 1, ten plants per plot were dug to a depth of 20 cm; number of nodules and number of pink nodules were rated on each plant dug, according to Rice *et al.* (1977).

In Expts 2 and 3, nodulation was assessed on ten plants according to the British Columbia Ministry of Forests field guide (BCMF 1991) where a ranking of 0 or 1–5 is given to each of five categories and a sum is taken: plant vigour (1 for very chlorotic plants to 5 for green, vigorous plants); nodule number (0 for no nodules to 5 for 5–50 effective nodules); nodule position (1 for nodules on lateral roots only to 5 for nodules predominately on the crown); nodule colour (0 for white or green to 5 for predominantly pink); and nodule appearance (0 for ineffective to 5 for effective).

Evaluation of DM yield and CP

A Mott flail type harvester was used to harvest the central two rows (0·6 × 5·5 m) of each plot, leaving 7·5 cm stubble. The wet weight of the whole harvest zone was determined in the field and a 500 g subsample was oven-dried at 55 °C to adjust for moisture content. In Expts 2 and 3, DM samples were ground to pass through a 1-mm mesh Wiley mill and the total N content determined (Nelson & Sommers 1980).

Soil measurements

In the autumn of 1989, surface soil samples (0–15) from Expt 1 were collected from each of the four main plots, with and without lime, to determine the effect of liming on the nutrient availability of the soil. Samples were air-dried and passed through a 2 mm sieve prior to chemical analysis. Samples were analysed for calcium (Ca), magnesium (Mg), potassium (K), nitrate-nitrogen ($N\text{-}NO_3$), phosphorus (P), sodium (Na), sulphur (S) and organic matter (OM) and pH by the Griffin Laboratories, Kelowna, British Columbia. Ca, Mg, Na, P and S were extracted using the Kelowna multi-element extractant (0·25 N HOAc and 0·015 N NH_4F at 1:10 soil:solution v/v with a 5-min extraction time) (van Lierop 1986, 1989). Organic matter was determined by colorimetry using the Walkley-Black method (Walkley & Black 1934), while pH was determined in water (Hendershot *et al.* 1993).

For the experiments sown in 1992, soil samples were collected from Expt 2 at three depths (0–7·5, 7·5–15 and 15–30 cm) during the autumn of 1994, air-dried and passed through a 2-mm sieve prior to determining soil pH in water (Hendershot *et al.* 1993).

Statistics

All results and data were subjected to analyses of variance (ANOVA) using PROC GLM (SAS 1985). Significance was tested at $P \leqslant 0{\cdot}05$.

RESULTS

Experiment 1

The clovers had significantly more nodulated plants, more nodules per plant and higher nodule colour

Table 2. *Effect of preinoculation and legume species on nodulation rating for the three legume species. Sample size was 8 for individual seed treatments, or 16 for both treatments*

Species	Bare seed	Preinoculated seed	Both
	Percentage plants nodulated		
Lucerne	18	39	28
Red clover	89	94	91
Alsike clover	86	89	88
S.E.	6·6*		4·7
D.F.	30		30
	Nodule number rating (0–3)		
Lucerne	0·2	0·4	0·3
Red clover	1·6	1·4	1·5
Alsike clover	1·5	1·3	1·4
S.E.	0·11*		0·08
D.F.	30		30
	Nodule colour rating (0–4)		
Lucerne	0·0	1·3	0·7
Red clover	2·7	3·1	2·9
Alsike clover	2·4	2·9	2·6
S.E.	0·23		0·16
D.F.	30		30

* Interaction effect not significant.

Table 3. *Effect of preinoculation, lime and legume species on dry matter yield (t ha^{-1}) of legumes sown 9 June 1989. The 1990 and 1991 cut 1 values from all three species are means from* n = *16 for species and 24 for inoculation. The 1991 and 1992 values for total yield from lucerne are means for* n = *8 for inoculation effect and* n = *4 for interaction effect*

	All species		Lucerne	
	1990 Total	1991 Cut 1	1991 Total	1992 Total
Preinoculation effect				
Seed bare	3·06	1·12	3·78	1·93
Seed preinoculated	4·00	2·07	4·95	1·84
S.E.	0·187	0·095	0·388	0·181
D.F.	30	30	6	6
Species effect				
Peace lucerne	2·12	3·19	—	—
Alsike clover	4·18	0·73	—	—
Red clover	4·29	0·87	—	—
S.E.	0·228	0·116	—	—
D.F.	30	30		
Interaction effect				
Bare, without lime	2·87	0·81	2·82	1·19
Bare, with lime	3·25	1·44	4·74	2·92
Preinoculated, no lime	3·78	1·85	5·08	1·94
Preinoculated, plus lime	4·22	2·28	4·81	1·73
S.E.	0·457*	0·232*	0·323	0·730
D.F.	30	30	6	6

* Interaction effect not significant.

rating than lucerne (Table 2). Preinoculation did not significantly change the number of plants nodulated or number of nodules per plant. In contrast to the clovers, lucerne had effective pink nodules, indicated by a higher colour rating, only when seed was preinoculated.

Liming at 2·3 t ha^{-1} had no effect on any of the three nodulation measurements. Nevertheless, analysis of soil sampled in the autumn of 1989 showed that liming increased the concentration of Ca from 629 to 1164 mg kg^{-1} (S.E. = 285·0) and of Mg from 526 to 640 mg kg^{-1} (S.E. = 50·7) and increased pH from 5·2 to 5·4 (S.E. = 0·13). The availability of N-NO_3, P, K, Na and S and the level of OM were not affected by the application of lime.

Plant establishment in the sowing year (1989) was higher for lucerne than for the clovers. For lucerne, 70% of the seeds sown established, while for alsike clover and red clover, 38 and 47%, respectively, successfully established, (S.E. = 4·8).

Plant survival decreased during the first year for all three species, with alsike clover showing the largest decrease. In 1990, 34, 9 and 36% of lucerne, alsike and red clover seeds sown, respectively, were present as established plants (S.E. = 3·4). During the winter of 1990/91, the clovers were severely winter-killed.

For 1990, the year following sowing, clovers yielded more DM ha^{-1} than lucerne (Table 3). However the

Table 4. *Effect of lime and preinoculation on nodulation rating (potential total is 25) of lucerne lines sown in 1992. Values are means from* n = *18 for the no lime preinoculated treatment and* n = *12 for the other three treatments*

Lime	Preinoculated	1992 (7 Oct.)	1993 (8 June)
No	No	9·0	7·0
	Yes	17·6	17·2
Yes	No	18·0	16·8
	Yes	20·1	18·8
S.E. ($n = 12$)		1·00	0·75
S.E. ($n = 18$)		0·82	0·61
D.F.		32	32

clovers yielded only 25% as much DM as lucerne for cut 1 in 1991 (Table 3) owing to the winter-kill that had occurred in the clovers. Seed preinoculation increased 1990 DM yield and 1991 cut 1 DM yield. Liming the soil, at 2·3 t ha^{-1}, did not significantly increase 1990 or 1991 cut 1 DM yield. When only lucerne DM yield was evaluated in 1991 and 1992, a lime × seed preinoculation effect was detected (Table 3). In the absence of lime, preinoculation increased DM yield, but in the presence of lime, DM yield was

Table 5. *Effect of lime and inoculation on 1993 DM yield (t ha*$^{-1}$*) and percentage CP for lucerne lines sown in 1992. Values are means from* n = *18 for the no lime preinoculated treatment and* n = *12 for the other three treatments*

Lime	Preinoculated	Cut 1 (8 July)	Cut 2 (7 Sept)	Total
		DM yield		
No	No	1·40	0·83	2·22
	Yes	2·47	1·83	4·30
Yes	No	3·28	2·10	5·38
	Yes	3·22	1·98	5·21
S.E. (n = 12)		0·140	0·125	0·242
S.E. (n = 18)		0·114	0·102	0·198
D.F.		32	32	32
		percentage CP		
No	No	9·2	17·7	—
	Yes	12·9	18·5	—
Yes	No	17·1	18·0	—
	Yes	16·0	18·8	—
S.E. (n = 12)		0·53	0·39	—
S.E. (n = 18)		0·44	0·32	—
D.F.		32	32	

Table 6. *Effect of lime and rhizobial strain on nodulation rating (potential total is 25) of lucerne lines sown in 1992. Values are means for* n = *9*

Lime	Inoculum strain	1992 (8 Oct.)	1993 (9 Jun)
No	None	7·8	7·8
	NRG185	19·2	19·2
	GT004	23·0	22·9
	GT005	21·4	21·2
Yes	None	19·0	19·0
	NRG185	22·8	22·9
	GT004	23·4	23·4
	GT005	24·6	24·7
S.E.		1·13	1·30
D.F.		44	44

Table 7. *Effect of lime and rhizobial strain on 1993 DM yield (t ha*$^{-1}$*) and percentage CP from lucerne lines sown in 1992. Values are means for* n = *9*

Lime	Inoculum strain	Cut 1 (July 9)	Cut 2 (Sept 8)	Total
		DM yield		
No	None	1·30	0·80	2·10
	NRG185	2·29	1·84	4·12
	GT004	2·50	1·65	4·15
	GT005	2·72	1·92	4·64
Yes	None	2·70	1·82	4·60
	NRG185	3·10	1·94	5·09
	GT004	2·56	1·91	4·47
	GT005	3·16	2·12	5·29
	S.E.	0·259	0·116	0·335
	D.F.	42	44	42
		percentage CP		
No	None	9·2	16·5	—
	NRG185	11·9	16·6	—
	GT004	12·7	16·8	—
	GT005	12·8	16·5	—
Yes	None	15·5	16·5	—
	NRG185	15·5	16·6	—
	GT004	14·5	16·5	—
	GT005	14·1	16·6	—
	S.E.	0·45	0·38	—
	D.F.	42	44	

not affected by preinoculation. This lucerne × lime × preinoculation effect appeared to be present earlier but was masked when all species were analysed together.

Experiments 2 *and* 3

Liming at 5 t ha^{-1} increased soil pH in Expt 2. Soil pH at three depths, 0–7·5, 7·5–15 and 15–30 cm, for samples taken during the autumn of 1994 were 5·0, 5·1 and 5·2 for the no-lime treatment and 5·6, 5·4 and 5·4 for the limed treatment. Preinoculation increased nodulation rating for the no-lime treatment but not for the limed treatment (Table 4). Root nodulation was similar for the six lucerne synthetic lines included in Expt 2. Preinoculation increased DM yield and percentage CP in the absence of lime, but not in the presence of lime, in the year following sowing (Table 5).

As observed in the first two experiments, preinoculation increased root nodulation rating in the absence of lime but not in the presence of lime in Expt

3 (Table 6). Root nodulation was similar for the three rhizobia strains. In the absence of lime, preinoculation increased DM yield more than in the presence of lime (Table 7). Preinoculation increased the %CP of lucerne without lime at cut 1 but not at cut 2. The only rhizobia strain effect observed was that GT005 gave a higher DM yield than GT004 in the presence of lime. Nevertheless, no strain was clearly better than the commercially available NRG185 strain.

Based on Expts 2 and 3, the treatment of adding only lime to the soil at a rate of 5 t ha^{-1} increased DM yield by an average of 130% and CP of cut 1 from 9·2 to 16·3%. Results from the inoculation treatment only, showed that DM yield increased by 100% and CP of cut 1 increased from 9·2 to 12·7%. As expected, lime and inoculation combined had a larger effect than either treatment alone. In this case, DM yield increased by an average of 136% and CP of cut 1 increased from 9·2 to 15·4%.

DISCUSSION

Because lucerne and clover plants had nodules in the absence of preinoculation, a 'native' population of rhizobia resides in the soil that can infect all three species. But for the formation of effective pink nodules that could fix atmospheric N, it was necessary to apply inoculum with lucerne seed but not with clover seed. These lucerne nodulation results are consistent with those obtained in northern Alberta, where a soil with a pH of 5·8–5·9 had $3·3 \times 10^6$ ineffective rhizobia g^{-1} and only 10^2 effective rhizobia g^{-1} (Rice & Olsen 1983).

Despite the lower DM yield from lucerne compared to the clovers during the year following sowing, lucerne persisted longer. Stand thinning with age is typical of legume stands such as lucerne (Tesar & Marble 1988), while alsike clover is known to be a short-lived legume (Heath *et al.* 1973). Because of longer persistence, the average DM yield over a period of years will be higher for lucerne than for clovers in the absence of reseeding. For this reason, further work on the adaptability of lucerne to moderately acid soil was undertaken.

Neither the six lucerne lines, nor the three rhizobia strains tested, improved nodulation or yield under moderately acidic conditions. The absence of a genotype effect among cultivars tested is consistent with results reported by Buss *et al.* (1975). They reported a narrow range of genetic variability for growth of lucerne in acid soils.

Two results were obtained in each of the three experiments. First, when lime was incorporated into the soil, supplying inoculum with the seed had only a small additional effect. Second, sowing coated preinoculated seed in the absence of soil liming resulted in good nodulation of lucerne roots in soil with a pH of 5·0–5·20. Munns (1970) also reported that lucerne can be nodulated at pH $\geqslant$ 5·2 Our results at pH 5·0–5·2 support those of Rice & Olsen (1983) who reported that the use of coated seed was an effective method of inoculating lucerne sown in soil with pH 5·8–5·9

Although the yield increase from preinoculation alone was lower than the increase from lime alone, preinoculation might be the preferred method based on cost. At current prices, it costs \$39·85 ha^{-1} more to sow coated preinoculated Peace seed than to sow bare Peace seed. Liming at a rate of 2–5 t ha^{-1} would add \$108·00–270·00 to the cost of sowing 1 ha. Therefore, liming costs from 2·5 to 7 times as much as using lime-coated preinoculated seed.

Although less convenient to use than lime-coated preinoculated seed, on-farm field inoculation would cost less (only *c.* \$0·17 kg^{-1} of seed, for inoculum and sticker, plus labour). However, additional research is required to determine if the field inoculation procedure is as effective as using preinoculated lime-coated seed.

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REFERENCES

AGRICULTURE CANADA (1991). The method of testing legume inoculant and preinoculated seed products. *Fertilizers Act, Section 23, Regulations.* Ottawa, Ontario: Canada Department of Agriculture.

BRITISH COLUMBIA MINISTRY OF FORESTS (1991). *Field Guide to Nodulation and Nitrogen Fixation Assessment. Land Management Handbook, Field Guide Insert 4.* Smithers, British Columbia: BCMF.

BROERSMA, K. (1987). Acid soils in western Canada. In *Proceedings of the Western Region Workshop on Forage Crops* (Ed. G. P. Goplen), pp. 87–120. April 6–8, 1987, Calgary, Alberta.

BUSS, G. R., LUTZ, J. A. & HAWKINS, G. W. (1975). Yield response of alfalfa cultivars and clones to several pH levels in Tatum subsoil. *Agronomy Journal* **67**, 331–334.

CANADA SOIL SURVEY COMMITTEE (1978). *The Canadian System of Soil Classification.* Canadian Department of Agriculture, Publication 1646. Ottawa, Ontario: Supply and Services Canada.

FARSTAD, L. & LAIRD, D. G. (1954). *Soil Survey of the Quesnel, Nechako, Francois Lake and Bulkley-Terrace Areas in the Central Interior of British Colombia.* Report No. 4 of the British Columbia Soil Survey. Experimental Farms Service, CDA. Ottawa: Queens Printer.

HEATH, M. E., METCALFE, D. S. & BARNES, R. E. (1973). *Forages: The Science of Grassland Agriculture*. Ames, Iowa, USA: The Iowa State University Press.

HELY, F. W. (1965). Survival studies with *Rhizobium trifolii* on seed of *Trifolium incarnatum* L. inoculated for aerial sowing. *Australian Journal of Agricultural Research* **16**, 575–589.

HENDERSHOT, W. H., LALANDE, H. & DUQUETTE, M. (1993). Soil reaction and exchangeable acidity. In *Soil Sampling and Methods of Analysis* (Ed. M. R. Carter), pp. 141–146. Boca Raton, Florida, USA: Lesi Publishers.

LAVKULICH, L. M. (1980). The agricultural potential of soils in central British Columbia. In *Proceedings of the Symposium on the Agricultural Potential of Central British Columbia*, pp. 4–12. University of British Columbia and the College of New Caledonia.

MUNNS, D. N. (1970). Nodulation of *Medicago sativa* in solution culture V. Calcium and pH requirements during infection. *Plant and Soil* **32**, 90–102.

NELSON, D. W. & SOMMERS, L. E. (1980). Total nitrogen analysis in soil and plant tissues. *Journal of the Association of Official Analytical Chemists* **63**, 770–778.

OLSEN, P. E. & RICE, W. A. (1989). *Rhizobium* strain identification and quantification in commercial inoculants by immunoblot analysis. *Applied and Environmental Microbiology* **55**, 520–522.

RICE, W. A. & OLSEN, P. E. (1983). Inoculation of alfalfa seed for increased yield on moderately acid soil. *Canadian Journal of Soil Science* **63**, 541–545.

RICE, W. A., PENNEY, D. C. & NYBORG, M. (1977). Effects of soil acidity on rhizobia numbers, nodulation and nitrogen fixation by alfalfa and red clover. *Canadian Journal of Soil Science* **57**, 197–203.

SAS INSTITUTE (1985). *SAS User's Guide: Statistics, 5th edn*. SAS Inst., Cary, North Carolina, USA.

SOIL SURVEY STAFF (1975). *Soil Taxonomy. USDA Handbook No. 436*. Washington, DC: United States Government Printing Office.

STOUT, D. G., HALL, J. W., BROOKE, B. M., BAALIM, G. & THOMPSON, D. J. (1993). Effect of storage temperature and time on viability of rhizobia on lime-coated alsike clover (*Trifolium hybridum*) seed. *Journal of Agricultural Science, Cambridge* **120**, 205–211.

TESAR, M. B. & MARBLE, V. L. (1988). Alfalfa establishment. In *Alfalfa and Alfalfa Improvement* (Eds A. A. Hanson, D. K. Barnes & R. R. Hill), pp. 303–332. Madison, Wisconsin, USA: American Society of Agronomy.

VAN LIEROP, W. (1986). Soil nitrate determination using the Kelowna multiple element extractant. *Communications in Soil Science and Plant Analysis* **17**, 1311–1329.

VAN LIEROP, W. (1989). Effect of EDTA and DTPA on available-P extraction with the Kelowna multiple element extractant. *Canadian Journal of Soil Science* **69**, 191–197.

WALKLEY, A. & BLACK, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **34**, 29–38.