

Caucasian clover responses to fertiliser, lime and rhizobia inoculation at Lake Heron Station, Canterbury

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Abstract

The agronomic performance of Caucasian clover in high country grasslands was the subject of two experiments at Lake Heron Station, Canterbury. In the first experiment, Caucasian clover was direct drilled into an undeveloped pasture (soil pH = 5.5; Olsen P = 7 mg/litre) with fertilisers containing similar P and S but \pm 20 kg/ha of N, in December 2011. After 11 months, shoots of Caucasian clover were small (3 mg), indicating an inoculation failure, and effects of fertilisers were not biologically meaningful. Therefore, the influence of rhizobia inoculant, superphosphate and lime on early growth of Caucasian clover was assessed using the same soil in a glasshouse. Un-inoculated plants were 13–24% the size of inoculated plants and this lack of vigour was not overcome by fertilisers. In the second field experiment, an established Caucasian clover/browntop pasture (soil pH = 5.5; Olsen P = 5 mg/litre) received 0, 100, 200 and 400 kg/ha of superphosphate with 0 or 5 t/ha of lime in February 2012. Despite the high inputs, spring pasture yields 9 and 21 months after fertiliser application were low (1260–2400 kg DM/ha), but the contribution of Caucasian clover was high (66–76%).

Keywords: high country, nitrogen, phosphorus, *Trifolium ambiguum*

Introduction

Caucasian clover (*Trifolium ambiguum* M. Bieb) is a persistent legume option for high country grasslands in New Zealand (Allan & Keoghan 1994; Scott 1998; Strachan *et al.* 1994; Woodman *et al.* 1992). As with most pasture legumes, its establishment and persistence is dependent on its ability to fix atmospheric nitrogen (N) via symbiotic bacteria (“rhizobia”) in root nodules. This ability can help the plant compete against non-legume plants under low soil N conditions. However, the rhizobia suitable for Caucasian clover must be inoculated on to the seed before sowing, which can be difficult in practice (Pryor *et al.* 1998), and little N may be fixed in the first year (Seguin *et al.* 2001). Establishment of Caucasian clover in tussock grasslands is improved when inoculated seed is direct drilled with fertilisers that contain phosphorus (P)

and sulphur (S) (Lucas *et al.* 1980; Moorhead *et al.* 1994). Ongoing agronomic performance of Caucasian clover is also dependent on use of P and S fertilisers (Scott 1998). However, there is limited information on establishment of Caucasian clover by direct drilling with fertilisers containing N, and about the fertiliser and lime inputs required for Caucasian clover to thrive in the high country.

Therefore, two field experiments were carried out at Lake Heron Station, Canterbury, and a glasshouse experiment at Lincoln University, in which the objectives were to:

- 1) determine if N fertiliser can improve the establishment of Caucasian clover when direct drilled into undeveloped tussock grassland,
- 2) assess the role of rhizobia inoculation, superphosphate and lime on early growth of Caucasian clover under glasshouse conditions,
- 3) determine the superphosphate and lime responses of established Caucasian clover in the South Island high country.

Methods

Field experiment 1: influence of N fertiliser on establishment of Caucasian clover

The first field experiment was conducted in an undeveloped (“native”) pasture at Lake Heron Station, Canterbury (43°29'27.08"S, 171°13'11.61"E). The soil was a “Cass Series” High Country Southern Brown soil (New Zealand Classification: Upland Allophanic Brown Soil (Hewitt 1998); USDA: Dystrachrept (Soil Survey Staff 1998)). An initial soil test indicated: pH = 5.5, Al (CaCl₂ extractable) = 2.1 mg/kg, Olsen P = 7 mg/litre, sulphate S = 14 mg/kg, Quick Test (QT) Mg = 16 and QT K = 10. The site is 765 m above sea level, flat and with an estimated annual rainfall of 700–800 mm.

The resident pasture was depleted fescue tussock (*Festuca novae-zelandiae* (Hack) Ckn.) dominated by browntop (*Agrostis capillaris* L.), Kentucky bluegrass (*Poa pratensis* L.) and sweet vernal (*Anthoxanthum odoratum* L.) grasses and mouse-ear hawkweed (*Hieracium pilosella* L.) with low (<1%) legume content and up to 30% bare ground. The site has had no regular fertiliser or lime inputs.

The pasture was sprayed with Glyphosate 360 (2 litres/ha, 36% a.i.), grazed by sheep and then divided into six large plots (ca. 2 ha) in preparation for sowing. On 20 December 2011, the plots were drilled with a seed mix of 'Endura' Caucasian clover (6 kg/ha of commercially inoculated coated seed) and 'Vision' cocksfoot (*Dactylis glomerata* L.) (2 kg/ha) and one of two fertilisers using a Duncan Enviro 3000E triple disc drill, according to a randomised block design with three replicates. The two fertilisers were superphosphate (9% P and 12% S) at 110 kg/ha and Cropmaster 20 (20% N, 10% P and 12.5% S) at 100 kg/ha. Hence all plots received similar rates of P and S, but the Cropmaster 20 plots received 20 kg/ha of N. Sulphur Super 20 (8% P, 20.6% S) was applied across all plots at 250 kg/ha in February 2012. The pasture was neither grazed nor fertilised over the following 12 months.

Caucasian clover plants were assessed for population and shoot dry weight 11 months after sowing (28 November 2013). Plants were sampled from five random 2 m lengths of drill row per plot.

Glasshouse experiment: assessing the role of rhizobia inoculation, superphosphate and lime on early Caucasian clover growth

The glasshouse experiment was conducted at the Lincoln University Nursery. Treatments were all possible combinations of the presence and absence of rhizobia inoculant, superphosphate and lime. Each combination was replicated four times according to a 2³ factorial in a randomised block design with 1.3 litre plastic pots.

Soil (0–0.2 m depth) was collected in March 2013 from a site next to the first field experiment at Lake Heron Station. The soil was mixed, sieved and analysed (pH = 5.4, Al = 6.4 mg/kg, Olsen P = 7 mg/litre, sulphate S = 9 mg/kg, QT Mg = 9 and QT K = 7). It was then mixed with the appropriate rates of superphosphate and lime and placed into pots. The equivalent to 200 kg/ha of superphosphate (1.1 g of monobasic phosphate monohydrate and 0.5 g of gypsum/pot) and 3 t/ha of lime (2.8 g of calcium carbonate/pot) were used.

'Endura' Caucasian clover seeds were inoculated with the *Rhizobium leguminosarum* bv. *trifolii* strain CC283b (Becker Underwood, Australia), a currently recommended inoculant for *Trifolium ambiguum* in New Zealand, within 2 days before planting into each pot.

Pots were watered with tap water every 1–2 days. Plants were gradually thinned to three per plot. These remaining plants were harvested 19 weeks after sowing and assessed for shoot and root dry weights and number of root nodules. For each superphosphate × lime treatment, bulk soil samples were analysed for Olsen P and sulphate S, and bulk shoot samples of inoculated Caucasian clover plants were analysed for P, S and N.

Field experiment 2: superphosphate and lime responses of established Caucasian clover

The second field experiment made use of a 10-year-old pasture of Caucasian clover (43°23'31.47"S, 171°9'5.89"E) on a similar soil to the first field experiment. The site is 680 m above sea level with an estimated annual rainfall of 900 mm, although summer droughts and dry winds are common on this moderately flat and exposed site. The Caucasian clover cultivar was 'Endura' and it was drilled in 2002. There had been no fertiliser inputs since an application of 150 kg/ha of superphosphate at sowing.

Superphosphate (9% P and 12% S) was broadcast on to the pasture at four rates (0, 100, 200 and 400 kg/ha) in the absence and presence of lime (5 t/ha) in February 2012, according to a 4 × 2 factorial in a randomised block design with four replicates. Plot size was 12 × 20 m.

Pasture production was measured 9 and 21 months after fertiliser application (28 November 2012 and 21 November 2013) in a random site in each plot. At each site a 5 × 0.46 m strip was mown to 2 cm above ground. The sample was weighed and a 100 g subsample dried at 70°C to determine dry matter (DM) content, which was applied to whole sample fresh weight to calculate total yield (kg DM/ha). Another sample was clipped from beside the mown strip, separated into Caucasian clover and other species, and dried at 70°C to determine Caucasian clover yield. Pasture production was also observed twice over summer and autumn each year, but on each occasion the pasture was only 2–3 cm tall and therefore impractical to harvest.

Plots were grazed with Merino sheep for 2–3 days soon after sampling in November and in April/May each year.

Soil samples (0–7.5 cm depth) were taken across the area at the start of the experiment, and from each plot (15 cores/plot) 9 months after fertiliser application (28 November 2012). The plot samples were bulked on treatment. All samples were analysed for Olsen P, sulphate S, pH and Al (Hill Laboratories, Hamilton).

Lamina plus petiole samples of Caucasian clover were taken from each plot 9 months after fertiliser application (28 November, 2012) for analysis of P and S contents (Lincoln University).

Caucasian clover was assessed for number of shoots and taproots, and rhizome and root mass 25 months after fertiliser application (19 March 2014). A random 0.38 × 0.38 m quadrat was dug to 0.10 m depth in each plot that received the lowest and highest rates of superphosphate and lime. Caucasian clover was separated from other species, washed and assessed before drying at 70°C.

All response variables in the three experiments were tested for significant ($\alpha=0.05$) treatment effects and interactions by analysis of variance using GenStat 16 statistical software.

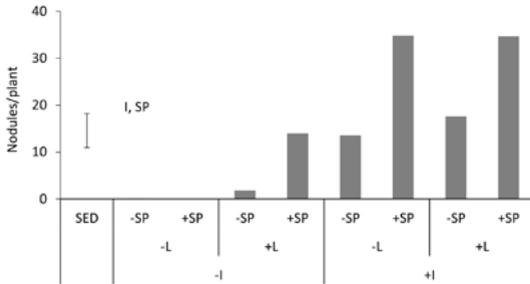


Figure 1 Nodulation of Caucasian clover in response to rhizobia inoculation (I), superphosphate (SP) and lime (L), 19 weeks after treatment under glasshouse conditions in a soil from Lake Heron Station, Canterbury. Standard error of difference is given as the vertical bar and the occurrence of significant ($\alpha=0.05$) treatment effects are indicated.

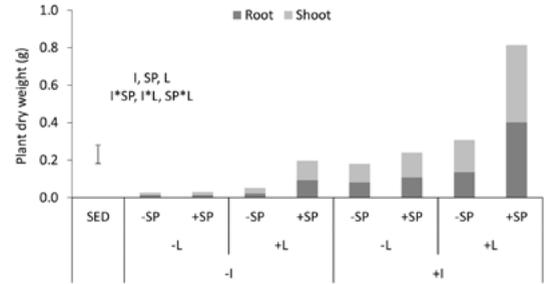


Figure 2 Growth of Caucasian clover in response to rhizobia inoculation (I), superphosphate (SP) and lime (L), 19 weeks after treatment under glasshouse conditions in a soil from Lake Heron Station, Canterbury. Standard error of difference for total plant weight is given as the vertical bar and the occurrence of significant ($\alpha=0.05$) treatment effects are indicated.

Results and Discussion

Field experiment 1: influence of N fertiliser on establishment of Caucasian clover

After 11 months, the biomass of Caucasian clover was small – shoots averaged 3 mg/plant and plant population averaged 37/m² – indicating poor early vigour. Given this, any effects of fertilisers up to this time were not biologically meaningful. However, these data are important as lack of early vigour of Caucasian clover is a key constraint to establishment, and this cannot always, as shown here, be overcome by use of fertilisers. Understanding this is key to future success of Caucasian clover and, as such, a glasshouse experiment was conducted to assess the role of rhizobia inoculation, superphosphate and lime on early plant growth.

Glasshouse experiment: assessing the role of rhizobia inoculation, superphosphate and lime on early Caucasian clover growth

Nutrient contents of the soil and shoots of the inoculated Caucasian clover plants are given for the

Table 1 Nutrient contents of soil and shoots of inoculated Caucasian clover plants in response to superphosphate (SP) in the glasshouse experiment.

	-SP	+SP
	Soil	
Olsen P (mg/litre)	5	37
Sulphate S (mg/kg)	14	99
	Shoots	
P%	0.27	0.37
S%	0.28	0.25
N%	4.40	4.39

All samples were bulked on treatment.

superphosphate treatments in Table 1. Soil pH was 6.2 and Al was <0.5 mg/kg for the limed pots compared with pH = 5.5 and Al = 4.4 mg/kg for the un-limed pots. After 19 weeks, the average number of nodules on inoculated Caucasian clover was 25/plant compared with ($P<0.001$) four/plant when un-inoculated (Figure 1). Growth of inoculated plants averaged 0.39 g compared with ($P<0.001$) 0.08 g for un-inoculated plants (Figure 2), and shoot N of inoculated plants was optimal (Table 1). These results suggest that 1) the rhizobia inoculant was effective and 2) rhizobia that nodulate Caucasian clover were not present in the Lake Heron Station soil. Both explanations are in agreement with Pryor *et al.* (1998) who stated that Caucasian clover seedlings will not form functional nodules in New Zealand soils without an effective rhizobia inoculum. Because of this, effects of superphosphate and lime on nodulation and growth were dependent ($P<0.05$) on whether or not the seed was inoculated before planting. When the seed was inoculated, superphosphate increased nodulation and had the greatest effect on growth when lime was applied. In contrast, any effects of fertilisers on un-inoculated plants were not biologically significant.

Field experiment 2: superphosphate and lime responses of established Caucasian clover

The initial soil test at this site indicated moderate acidity (pH 5.5) and Al (1.7 mg/kg). Olsen P was low (5 mg/litre) but sulphate S (11 mg/kg), QT Mg (10) and QT K (8) were adequate.

After 9 months (Nov. 2012), Olsen P levels were still within the low range for pasture production (Cornforth & Sinclair 1982) and similar across the fertiliser treatments (Table 2). Herbage P and S contents of Caucasian clover were also low (Cornforth & Sinclair 1982) but increased ($P<0.05$) with increasing rates of superphosphate. Lime raised soil pH from 5.5 to 6.0

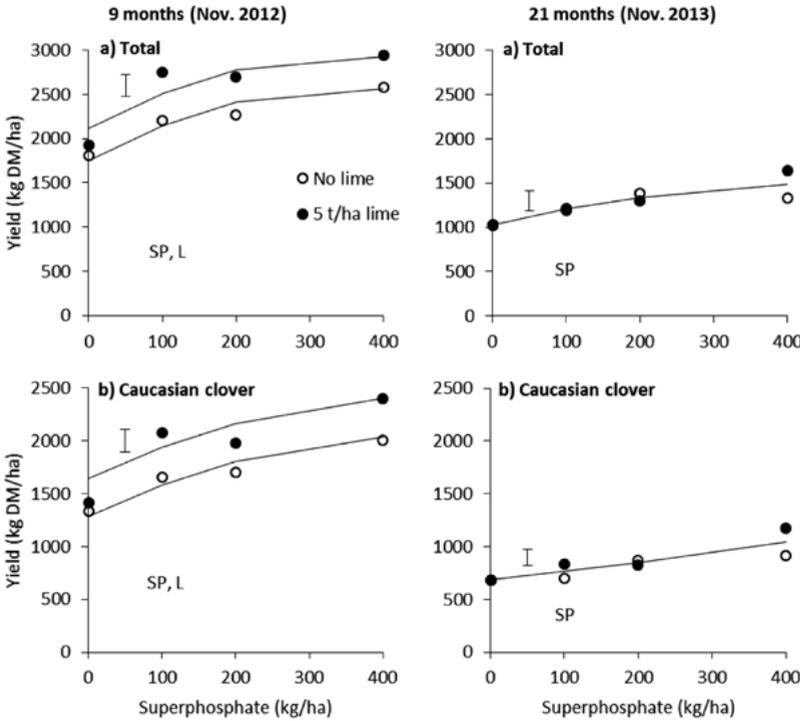


Figure 3 Dry matter yield of a) total herbage and b) Caucasian clover of an established Caucasian clover pasture in response to superphosphate (SP) and lime (L) at Lake Heron Station, Canterbury, 9 months (November 2012) and 21 months (November 2013) after fertiliser application. Standard error of difference is given as the vertical bar and the occurrence of significant ($\alpha=0.05$) treatment effects are indicated.

and lowered AI from 2.4 to 0.6 mg/kg, but had no effect on herbage P and S.

In November 2012 the average total yield was 2400 kg DM/ha (Figure 3). Caucasian clover contributed most of the yield (average 76%) and the balance was predominantly browntop. Total yield was affected ($P<0.05$) by superphosphate and lime. The superphosphate response was an average of 610 kg DM/ha for the 100 kg/ha rate and another 280 kg DM/

and taproots (9/m²), and rhizome and root mass (5.1 t DM/ha) of Caucasian clover. However, this below-ground biomass of Caucasian clover is a key advantage to persistence on infertile soils in extreme climates (Strachan *et al.* 1994).

Despite the high inputs of fertiliser, which are difficult and expensive to deliver to hill and high country systems, growth of Caucasian clover was low at this site. This result implies that climatic factors

ha at 400 kg/ha. This same response occurred when superphosphate was applied both with and without lime. Lime increased total yield by an average of 360 kg DM/ha. These effects appeared to be largely due to the stimulation ($P<0.05$) of Caucasian clover yield.

After 21 months (Nov. 2013), the average total yield was 1260 kg DM/ha and 66% of that was Caucasian clover (Figure 3). Superphosphate increased ($P<0.05$) total yield by an average of 460 kg DM/ha at the highest rate of 400 kg/ha, while lime had no effect. Again this effect of superphosphate was associated with a similar response ($P<0.05$) of Caucasian clover yield. Summer and autumn yields were negligible each year.

After 25 months (Mar. 2013), superphosphate and lime had no effect on numbers of shoot growing points (average 1620/m²)

Table 2 Phosphorus and sulphur contents in soil (0–7.5 cm) and herbage (lamina plus petiole) of established Caucasian clover in response to superphosphate at Lake Heron Station, Canterbury, 9 months (November 2012) after fertiliser application.

	Superphosphate (kg/ha)				P value	SED
	0	100	200	400		
Soil						
Olsen P (mg/litre)	11	11	11	14	†	-
Sulphate S (mg/kg)	8	10	9	10	-	-
Herbage						
P%	0.20	0.20	0.21	0.24	<0.001	0.006
S%	0.17	0.17	0.18	0.18	0.040	0.004

†Soil samples were bulked on treatment.

were the main constraints to legume production and thus 100–200 kg/ha of superphosphate appeared to be sufficient. However, the abundance of Caucasian clover in the pasture after 10 years (66–76% of spring yield) was higher than what would be expected from most other legume options (Woodman *et al.* 1992). This strong legume base could potentially be exploited to greater effect by spraying out the browntop and direct-drilling in a more productive grass such as cocksfoot.

Conclusions

Overall, the results suggest there was an inoculation failure in the first field experiment resulting in loss of Caucasian clover. This was not overcome by use of fertilisers and would be a costly outcome for a farmer. Therefore, establishment of Caucasian clover in high country grasslands in New Zealand appears to be initially dependent on overcoming issues associated with delivery of suitable rhizobia inoculants to the seed. At which point, ongoing agronomic performance should focus on delivery of suitable nutrients and overcoming any constraints associated with soil acidity.

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