Natural reseeding of caucasian clover (*Trifolium ambiguum*) in tussock grasslands

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Abstract

The potential of natural reseeding to increase the density of caucasian clover (*Trifolium ambiguum* M.Bieb.) swards in tussock grassland environments was investigated. Rates of pollination were high on all sites and viable seed was produced by mid March on sites ranging in altitude from 600 to 1100 m a.s.l. Hard seed, expected to survive passage through the grazing animal and germinate in the dung, was present. Rhizobia that effectively nodulate caucasian clover are absent from New Zealand soils and nodulation of seedlings and rhizome roots is dependent on spread of inoculant rhizobia in the soil. Lateral movement of rhizobia in soil did not exceed 6 cm per year, only slightly less than recorded rates of rhizome expansion. There was no evidence of movement of rhizobia in wind-blown dust or on sheep's hooves. Natural reseeding has the potential to 'thicken-up' swards of caucasian clover, but the slow spread of rhizobia indicates that nodulation failures will limit the extent of spread into unsown areas.

Keywords: caucasian clover, reseeding, rhizobia, rhizomes, *Trifolium ambiguum*

Introduction

Caucasian clover (*Trifolium ambiguum* M. Bieb.) is a rhizomatous perennial legume that shows promise as a persistent and productive legume for dry hill and high country (Woodman *et al.* 1992; Allan & Keoghan 1994). However, poor establishment following oversowing can result in swards with sparse numbers of caucasian clover plants (Lucas *et al.* 1981; Moorhead *et al.* 1994). The spread of established caucasian clover plants through rhizome growth (Allan & Keoghan 1994) and possibly through reseeding can increase sward density. Natural reseeding of white clover through seed ingestion and defecation by stock is a management tool for introducing white clover into new areas (Suckling 1950), but no information is available on seeding characteristics of caucasian clover in tussock grassland environments.

Rhizobia capable of effectively nodulating caucasian clover are absent from New Zealand soils (Patrick & Lowther 1995) and hence inoculation is essential. The success of natural reseeding will depend on colonisation of soil by the inoculant rhizobia. Lowther & Patrick (1993) concluded that the slow spread of rhizobia could affect the success of natural reseeding of lotus species. They also suggested that slow movement of rhizobia could limit vegetative spread of rhizomatous legumes, a suggestion supported by Scott & Mason (1992). They attributed the chlorotic shoots on the edge of a caucasian clover patch to a lack of nitrogen fixation owing to the rhizome growth exceeding the spread of the associated rhizobia. Under oversowing conditions only a small percentage (11–32%) of seeds germinate (Cullen & Ludecke 1967) and establishment of rhizobial populations from these ungerminated seeds is another possible means of rhizobia introduction.

This paper reports studies on seed production of caucasian clover over a range of tussock grassland environments, and on rhizobia establishment and movement in the soil, to determine the potential of natural reseeding.

Experimental

Seed production

Plots of caucasian clover differing in altitude, aspect and slope were selected in Otago tussock grasslands (Table 1). Monaro seed was oversown in spring 1992 and Treeline in spring 1993. The stage of the reproductive cycle (bud, inflorescence, post-flowering and seed) was recorded at approximately 3-weekly

Table 1 Details of sites containing established swards of cv. Monaro (6x) and cv. Treeline (4x) caucasian clover.

	Altitude (m a.s.l.)	Aspect (°)	Slope	pH	Date sown
cv. Monaro [1]					
Queensberry	970	NE	5	5.7	1992
Queensberry	760	SW	10	5.4	1992
Crown Range	1100	W	10	5.3	1992
Waitiri	860	SE	10	5.2	1992
Waitiri	800	NW	10	5.7	1992
Earnscleugh	600		flat	5.9	1991
cv. Treeline [2]					
Waitiri	950	NE	5	5.3	1993
Waitiri	860	SE	10	5.2	1993

[1] Patrick & Lowther 1995
[2] Patrick *et al.* 1994

intervals from early February until late April 1996. At least 10 caucasian clover flowering shoots were selected at random in each of four replicates. At the first sampling the number of florets per inflorescence was counted while the proportion of florets containing seed was assessed at the last sampling. Germination of seed collected in mid March was assessed by placing seed on moistened filter paper, chilling for 4 days at 4°C, and then incubating at 25°C. Remaining ungerminated seed was then scarified with sandpaper and re-incubated to determine germination from hard seed.

Rhizobia spread

Caucasian clover was planted in 1986 into a high country yellow-brown earth (pH 5.4) at Mt John. In January 1995, distinct foliage colour differences were observed similar to those reported by Scott & Mason (1992): the central area of the plant was dark green but foliage away from the central area was pale yellow. Soil samples (25 mm in diameter × 75 mm deep) were removed aseptically from the centre of the green foliage of 20 plants, on the edge of the green foliage, and 20, 40 or 60 cm from the edge, and the presence of rhizobia determined by the plant infection method (Brockwell 1963).

Caucasian clover seedlings were planted out in spring 1984, into a grazing trial on mid-altitude shady upland yellow-grey earth (pH 5.6) at Tara Hills (Allan & Keoghan 1994). In January 1994, soil and root samples were taken under established plants, and then rhizome and soil samples were taken at 15, 30, 45, 60 cm intervals from four plants at right angles to the slope. Soil samples only were taken at 75 and 90 cm as rhizomes were absent. The presence of rhizobia in the soil samples and on the rhizomes was determined.

In September 1992, inoculated Cau-casian clover was oversown onto a high country yellow-brown earth (pH 5.3) site on the Crown Range (Table 1). In February 1996, six replicate soil samples were taken aseptically on the edge of the plots, 2, 4, 6, 8 and 10 m downslope (10°) from the plots and the presence of rhizobia determined. At each position a soil core, 9 cm diameter × 10 cm deep, was also aseptically removed. Cores were placed in the glasshouse, sown with surface-sterilised seed and watered with sterile water when required. Seedlings were removed after 2 months and nodulation recorded to test for populations of rhizobia less than the four per g of soil detectable by the plant infection method.

Rhizobia establishment in the absence of the host

In September 1994, field experiments were laid down at both Hawkdun Station, St Bathans and at Eweburn Station, Naseby to determine rhizobia establishment where (A) seedlings failed to nodulate, and (B) seed did not germinate. Sites were on a high country yellow-brown earth with pH of 5.3 and 5.2 respectively. Experiment A: Caucasian clover seed inoculated at the normal rate and lime pelleted in the laboratory had 3 × 10^3 rhizobia per seed at oversowing. Seedlings at the cotyledon stage were marked. These were assessed 4 months later and soil samples 2.5 cm in diameter and 7.5 cm deep were taken where seedlings had failed to nodulate and the presence of rhizobia determined. Experiment B: Four replicates of 25 inoculated and pelleted seeds, where seeds had been killed by autoclaving prior to inoculation, were sown at marked locations. Soil samples were taken from below 10 of these seeds 9 and 12 months after sowing and the rhizobia presence determined. Also, at the 12 month sampling, surface-sterilised seeds were planted at the remaining five sites in each replicate, and seedlings were assessed for nodulation after a further 4 months to test for low populations of rhizobia.

Results

Seed production

At the beginning of February, caucasian clover plants on all sites had a mixture of immature buds, flowering and post-flowering stages. No seed was present at this assessment or in late February apart from some immature seed at the low Queensberry and Earnscleugh sites. By mid March, viable seeds were present at all sites. Between 35% and 66% of Monaro and 23% to 33% of Treeline florets contained seed (Table 2).

Table 2 Caucasian clover seed production on different sites (means ± standard deviation).

Sites	Altitude (m a.s.l.)	Florets per Inflorescence	Percentage of florets with seed	Initial germination (%)	Final germination (%)
cv. Monaro [1]					
Queensberry	970	116 ± 15.4	58 ± 30.0	13 ± 2.0	87 ± 10.0
Queensberry	760	143 ± 15.4	57 ± 12.8	8 ± 5.7	72 ± 20.4
Crown Range	1100	81 ± 7.0	66 ± 12.4	63 ± 18.3	87 ± 15.8
Waitiri	860	109 ± 20.0	55 ± 11.9	46 ± 13.3	95 ± 5.0
Waitiri	800	114 ± 17.5	35 ± 10.5	19 ± 11.5	87 ± 7.2
Earnscleugh	600	84 ± 8.1	60 ± 18.3	16 ± 4.0	87 ± 12.9
cv. Treeline [2]					
Waitiri	950	86 ± 12.4	33 ± 40.0	4[3]	100[3]
Waitiri	860	74 ± 10.8	23 ± 6.0	16[3]	88[3]

1 Patrick & Lowther 1995
2 Patrick *et al.* 1994
3 Replicates pooled

Germination varied widely over the sites, but with the exception of the Crown Range all sites had a high proportion of hard seed (Table 2). Seed viability was high, final germinations ranging from 72 to 100%.

Rhizobia spread

Caucasian clover rhizobia were detected in all samples taken from the centre and from the majority of samples taken at the edge of the dark green foliage of established caucasian clover plants at the Mt John site (Figure 1). Between 30% and 50% of samples taken under the yellow foliage had detectable populations of caucasian clover rhizobia.

Ten years after planting at the Tara Hills site, caucasian clover rhizobia were detected in all soil and rhizome samples up to 15 cm from established plants (Figure 2). Beyond this, rhizobia were detected in over half the soil samples up to 60 cm from the established plant but were not detected on any of the rhizomes. No rhizobia were detected in the soil at 75 and 90 cm.

Three years after oversowing at the Crown Range site, rhizobia were detected within the sown plot. However, rhizobia were not detected by the plant infection method in any of the soil samples taken from 2 to 10 m down slope. The absence of rhizobia was confirmed by the lack of nodulation on seedlings sown in soil cores taken from the same positions, apart from one of the cores taken at 6 m.

Rhizobia establishment in the absence of the host

High populations (>2.3×10^4/g soil) of caucasian clover rhizobia were detected under all nodulated plants at the Eweburn and Hawkdun sites, but there was little establishment of rhizobia in the soil in the absence of nodulated caucasian clover plants. Rhizobia were present in 8% and 18% of soil samples taken below non-nodulated seedlings 6 months after sowing at the Eweburn and Hawkdun sites respectively. Rhizobia were present in only 3% to 8% of soil samples taken below dead inoculated seeds 9 and 12 months after sowing at the two sites. All surface-sterilised seeds planted where the dead seeds had been sown failed to nodulate.

Figure 1 Presence of rhizobia in soil samples taken under caucasian clover at the Mt John site.

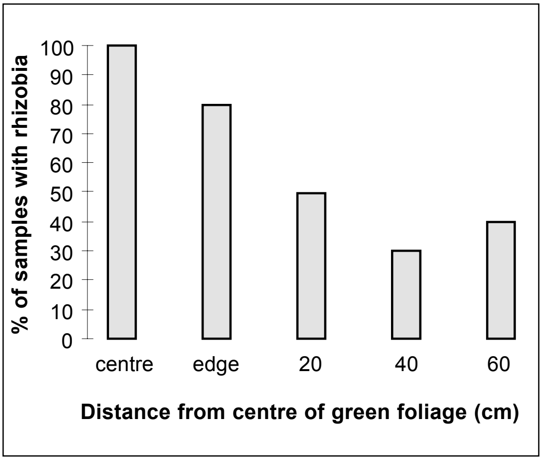
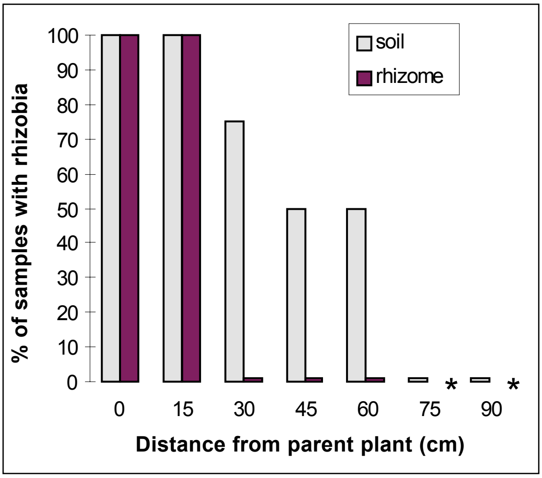


Figure 2 Presence of rhizobia on rhizome and in soil samples at measured distances from where caucasian clover was planted at the Tara Hills site. (* indicates absence of rhizomes).



Discussion

Caucasian clover produced viable seed on all sites chosen to cover the range of mid-altitude tussock grassland environments where it is expected to have agronomic potential (Allan & Keoghan 1994). Spelling blocks until mid-March should be sufficient to allow reseeding of caucasian clover. Hard seed of caucasian clover was present at each site, so germination after passage through the animal can be expected (Suckling 1952). There was no evidence that lack of pollination was a major limitation to seed production, as a high proportion of florets formed seed. Because all sites were surrounded by grazed tussock blocks, there was a possibility of preferential attraction of pollinators to the flowering caucasian clover in the plots; pollination rates in large blocks may therefore be lower than recorded under these trial conditions.

The absence of rhizobia in most soil samples under non-nodulated seedlings and below dead seed supports the conclusion of Lowther & Patrick (1993) that it would be unwise to assume widespread occurrence of rhizobia in the soil where seedling establishment failures have occurred. The inability of caucasian clover rhizobia to establish in the soil under the dead seed is surprising. Caucasian clover rhizobia are capable of multiplying and spreading, albeit slowly, in soil, but the present results suggest that rhizosphere stimulation, by exudates from the seedling root, is necessary for initial establishment. This result contrasts with Australian work with rhizobia nodulating *T. incarnatum* (Hely 1965) where, in the shade provided by tussocks or in weak sunlight, most of the inoculum came off the seeds and entered the adjacent soil as a result of the action of rain, dew and frost. Reasons for the lack of establishment of rhizobia in the soil in the present study are not clear.

The present results indicate that the chlorotic shoots on the expanding edges of caucasian clover plants (Scott & Mason 1992) cannot be attributed to rhizome growth exceeding rhizobia spread, as populations were detected in the soil under areas of chlorotic foliage. The maximum lateral spread of rhizobia recorded at Tara Hills was 6 cm per year. This exceeded the spread of rhizomes in the plots sampled but is similar to the 6.5 cm per year mean rhizome spread recorded on this site (Allan & Keoghan 1994). There was no evidence for movement of rhizobia with the rhizome. Rhizomes are underground stems and hence do not exhibit the rhizosphere stimulation of rhizobia that occurs around roots (Rovira 1961).

Movement of lotus rhizobia downslope has been shown to be more rapid than lateral movement, with rates of between 1 to 4 m per year (Lowther & Patrick 1993). However, at the Crown Range site no caucasian clover rhizobia were recorded 2 m downslope from established plants 3.5 years after sowing, indicating that caution must be used in estimating the extent of rhizobia spread downslope. The limited lateral spread of rhizobia after 10 years in the presence of sheep grazing confirms that movement in wind-blown dust and in soil on stock hooves plays only a small role in tussock grassland environments (Lowther & Patrick 1993). The presence of nodulated seedlings in one core 6 m from established plants on the Crown Range site indicates that sporadic transfer of rhizobia does occur.

Conclusions

Our results demonstrate that allowing natural reseeding is a potential management option to 'thicken-up' existing swards of caucasian clover in tussock grassland environments. The slow spread of rhizobia from established plants indicates the possibility of nodulation failure limiting the success of natural reseeding in spreading caucasian clover into unsown areas. However, it is not considered a limitation to vegetative spread. The occurrence of rhizobia will depend on initial establishment, topography, time since initial sowing and distance from established plants. Further study is required to determine the optimal conditions needed to achieve successful establishment of seedlings following natural reseeding.

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