

## Seedling establishment characteristics of alternative legume species in tussock grassland environments

W.L. LOWTHER AND HEATHER N. PATRICK  
*Invermay Agricultural Centre, AgResearch, Private Bag, Mosgiel*

### Abstract

Four alternative legume species, Caucasian clover (*Trifolium ambiguum*), zigzag clover (*T. medium*), crown vetch (*Coronilla varia*), and birdsfoot trefoil (*Lotus corniculatus*), were compared with white clover (*T. repens*) on four tussock grassland sites. Seed was inoculated and pelleted, and then **oversown** at a rate equivalent to 5 kg/ha viable seed. Germination, nodulation and survival of white clover, Monaro Caucasian clover, and zigzag clover were similar on the two lowest sites (600 and 830 m). However, low rates of zigzag clover germination occurred on the two higher sites (1090 and 1100 m). Germination of crown vetch appeared to be prevented or delayed by the low temperatures, and no plants established on any site. Low nodulation occurred in **Treeline** Caucasian clover and birdsfoot trefoil. Further research is required to select more suitable strains of rhizobia for both these legumes. The results provide information for estimating likely plant densities of these new cultivars when **oversown** in tussock grassland environments.

**Keywords** *Coronilla varia*, establishment, germination, inoculation, *Lotus corniculatus*, nodulation, *Trifolium ambiguum*, *Trifolium medium*, *Trifolium repens*

### Introduction

Limitations in the suitability of conventional legume species for some areas of the tussock grasslands have been identified by Allan (1985). In a survey on Tara Hills, Allan & Chapman (1987) reported that white clover (*Trifolium repens*) was the most successful clover but it had not persisted on sunny faces. Red clover (*T. pratense*) and alsike clover (*T. hybridum*) had not persisted in intensively managed swards. Caucasian clover (*T. ambiguum*), zigzag clover (*T. medium*), crown vetch (*Coronilla varia*) and birdsfoot trefoil (*Lotus corniculatus*) are legumes suggested as having potential for tussock grassland environments (Keoghlan 1985; Scott & Charlton 1983). However, most of the work investigating these species has been carried out with small plot

trials, sometimes with transplanted spaced plants. Little information is available on establishment of these new cultivars. In fact, Lucas *et al.* (1981) reported sparse establishment of Caucasian clover in contrast to white clover.

The aim of the present experiment was to obtain information on the germination, nodulation and establishment of these alternative legume species, compared with white clover, on a range of sites in the Otago tussock grasslands.

### Materials and methods

Seed of Caucasian clover (cvs Monaro and **Treeline**), zigzag clover (**G41**), crown vetch (**G32**), and white clover (cv Grasslands Huia) was inoculated with peat based inoculants at a high rate (30 g peat per kg seed) and pelleted using 40% gum arabic as the adhesive and microfine lime as a coating material. Birdsfoot trefoil (cv Maitland) seed was inoculated and coated by Coated Seed Ltd. Strains of rhizobia used were ICMP 4073b for Monaro Caucasian clover, 4074b for **Treeline** Caucasian clover, **8378b** for zigzag clover, **2153b** for white clover, 5058 for crown vetch and SU 343 for birdsfoot trefoil.

Species were oversown, at a rate equivalent to 5 kg/ha viable bare seed, on four undeveloped tussock grassland sites in the Mackenzie Basin and Central Otago in mid-September 1990. The species were randomised in four replicate blocks on each site. The Berwen-low site was on a yellow-grey earth soil with a pH (1:2.5 soil:water) of 5.9 at an altitude of 830 m. There was a thick vegetative cover of snow tussock (*Chionochloa rigida*), fescue tussock (*Festuca novae-zelandiae*), browntop (*Agrostis capillaris*), a native plant understorey and 20% bareground. The Berwen-mid site was on a yellow-brown earth soil with a pH of 5.6 at an altitude of 1090 m. The vegetation was similar to the low site but more sparse with 40% bare ground. The Tara site was on a YBE soil with a pH of 5.2 at an altitude of 1100 m. The predominant vegetation was snow tussock with 65% bare ground. The Earnscleugh site was on a brown-grey earth with a pH of 5.9 at an altitude of 600 m. The dominant vegetation cover consisted of fescue tussock, silver tussock (*Poa caespitosa*), hair grass (*Vulpia bromoides*) and 45% bare ground. A basal dressing of 200

kg/ha of molybdic-sulphur-superphosphate (0.01% Mo; 8% P; 19% S) was applied before sowing. Seed was **oversown** onto the undisturbed soil surface at all sites. However, on the semi-arid Eamscleugh site, seed was lightly raked into the soil surface to duplicate the effect of stock trampling in improving **seed:soil** contact (Allan *et al.* 1987). **Miral** (20 kg/ha) was applied prior to seed germination to reduce insect damage, particularly from broad nosed weevils (Barratt & Johnstone 1984). With large scale sowings insect damage can be reduced by applying insecticide to the seed coating.

The numbers of rhizobia on the seed at sowing were estimated by plate counts (Vincent 1970). Seed germination/early **seedling** establishment was assessed by **quadrat** counts when **seedlings** reached the cotyledon/unifoliate stage (6-10 weeks after sowing) and results have been expressed as a percentage of viable seed sown. At the same time, 20 seedlings in each plot were pegged **with** wire markers and the percentage of nodulated plants was recorded 17-21 weeks after sowing. Survival of pegged seedlings was assessed 8 and 14 months after sowing. Percentage seed germination at each site was **analysed** by analysis of variance according to the **randomised** block design. Seedling nodulation and survival out of 20 plants per plot at each site was analysed as a **generalised** linear model with binomial error distribution, fitting terms for block and species and calculating effective standard errors on the back transformed scale.

## Results and discussion

### Populations of rhizobia

Clovers and crown vetch had in excess of 3000 rhizobia per seed at sowing. These populations are higher than those shown to be necessary for the nodulation of white clover on a range of tussock grassland sites (Lowther 1977). The commercially pelleted birdsfoot trefoil contained 1100 rhizobia per seed at sowing, well in excess of the 300 per seed considered as the minimum standard for commercially pelleted white clover seed by the **Inoculant** and Coated Seed Testing Service (Johnson 1979).

### Germination and early seedling establishment

The percentages of seed germinating and reaching the cotyledon stage (germination) varied over the four sites (Table 1).

No crown vetch seedlings were found on the Berwen and **Tara** sites 6-8 weeks after sowing and there were only a low number at Eamscleugh after 10 weeks. Some crown vetch seedlings (< 1 per **m<sup>2</sup>**) were present on the **Berwen-low** site 17 weeks after sowing, indicating that

Table 1 Percentage of seeds germinating and reaching cotyledon stage, 6-10 weeks after sowing, relative to number of viable seed sown

	Berwen-low	Berwen-mid	Tara	Hills	Earnsdeugh
white	14	25	31	22	
<b>Monaro'</b>	<b>10</b>	<b>18</b>	40	31	
<b>Treeline'</b>	13	15	43	40	
<b>Zigzag</b>	10	5	<b>14</b>	24	
Birdsfoot	13	12	22	17	
Crown vetch	0	0	0	<b>11</b>	
<b>SED</b>	4.8	4.6	7.1	10.8	

'Caucasian clover

limited germination had occurred later in spring. Controlled environment studies have shown that crown vetch is not only slow to commence germination at optimum temperatures but also requires higher temperatures for **germination** than conventional clover **species** (Hill & Luck 1991). For example, the calculated base temperature for crown vetch (cv Chemung) germination was **6.9°C**, compared with **5.8°C**, **5.2°C** and **4.8°C** for white clover (cv Haifa), Caucasian clover (cv Monaro) and birdsfoot trefoil (cv Dewey) respectively. Hill & Luck (1991) concluded that crown vetch germplasm needs to be screened for better germination at low temperatures **to be** useful in temperate climates particularly when germination is required in early spring. This conclusion is supported by the present data.

There were no consistent differences in germination between the other species. However, there was a consistent trend for low germination from zigzag clover on the two highest sites.

Low rates of seed germination and early **seedling** establishment are major problems under oversowing conditions, particularly in drier environments, and trampling by stock hooves to improve **seed:soil** contact is well **recognised** as a means of improving establishment (Allan *et al.* 1987). **However, the technique is not widely practised** in the tussock grasslands because of the difficulty of obtaining sufficient stock in early spring to shepherd around **oversown** blocks. Similar germination rates to those obtained in the present study can therefore be expected when oversowing in **the** tussock grasslands.

### Nodulation

**No nodulated seedlings were recorded in crown vetch on any site.** Few healthy seedlings of any species were present on the dry Eamscleugh site due **to the early** onset of moisture stress.

Table 2 Percentage of seedlings that were nodulated, 17-21 weeks after sowing

	Berwen-low	Berwen-mid	Tara Hills	Earnsdeugh
White	5.0	3.5	2.5	9
Monaro <sup>1</sup>	4.4	4.6	4.3	6
Treeline <sup>1</sup>	1.5	1.9	1.4	0
Zigzag	4.6	4.6	2.6	6
Birdsfoot	4	1	5	0
Crown vetch	nd <sup>2</sup>	nd	nd	0 <sup>3</sup>
SED <sup>4</sup>	7.9	7.6	6.7	10.0

<sup>1</sup> Caucasian clover

<sup>2</sup> No data

<sup>3</sup> Not included in the statistical analysis

<sup>4</sup> Effective s.e. for differences from white clover

There was a virtual nodulation failure in birdsfoot trefoil on all sites. The commercially pelleted seed contained 1100 rhizobial seed at sowing, a number in excess of the standard for commercial pelleted seed in New Zealand (Johnson 1979). However, recent work (Patrick & Lowther 1993), has shown that even populations as high as 23000 rhizobia per seed are not sufficient to ensure nodulation of birdsfoot trefoil oversown onto difficult tussock grassland sites. Further research, in cooperation with commercial firms, is underway to alleviate the problem.

Seedling nodulation was low in Treeline Caucasian clover on all sites. Caucasian clover is very specific in *Rhizobium* requirements and strains effective on white clover do not form nodules or are ineffective on Caucasian clover (Parker & Allen 1952). In addition, different strains of rhizobia are required for tetraploid (eg Treeline) and hexaploid (eg Monaro) cultivars. The strains used for inoculation in the present study had been recommended after evaluation of nodulation and symbiotic effectiveness under controlled conditions. Selection of rhizobia for nodulating ability under oversowing conditions appears necessary to improve establishment of Treeline Caucasian clover.

Inoculation was carried out under laboratory conditions using a rate of peat inoculant (30 g per kg seed), higher than the rate (6.4-19 g) stipulated on commercial inoculants, to test the effectiveness of the strains of rhizobia. Further research is underway to define minimum populations of rhizobia needed for satisfactory nodulation of oversown Monaro Caucasian clover.

## Survival

On the two Berwen sites, survival in autumn (Table 3) and the following spring (Table 4) was closely related to the percentage of seedlings nodulated (Table 2). However, at Tara Hills, the majority of nodulated Caucasian clover and zigzag clover seedlings failed to survive. Due to the early onset of snow, the autumn assessment was not carried out at Tara Hills but visual observations indicated that there was a severe loss of seedlings over the summer due to moisture stress.

Table 3 Percentage plant survival 8 months after sowing, relative to initial seedlings established

	Berwen low	Berwen mid	Tara Hills	Earnsdeugh
White	61	46	nd <sup>2</sup>	5
Monaro <sup>1</sup>	31	55	nd	6
Treeline <sup>1</sup>	11	26	nd	21
Zigzag	54	40	nd	6
Birdsfoot	6	3	nd	3
Crown vetch	nd	nd	nd	0 <sup>3</sup>
SED <sup>4</sup>	6.1	6.0		3.8

<sup>1</sup> Caucasian clover

<sup>2</sup> No data

<sup>3</sup> Not included in statistical analysis

<sup>4</sup> Effective s.e. for differences from white clover

Table 4 Percentage plant survival 14 months after sowing, relative to initial seedlings established

	Berwen-low	Berwen-mid	Tara Hills	Earnsdeugh
white	61	46	2.0	9
Monaro <sup>1</sup>	35	59	14	4
Treeline <sup>1</sup>	14	29	4	14
Zigzag	51	41	8	6
Birdsfoot	6	3	0	0
Crown vetch	nd <sup>2</sup>	nd	nd	0 <sup>3</sup>
SED <sup>4</sup>	8.1	6.2	5.5	4.5

<sup>1</sup> Caucasian clover

<sup>2</sup> No data

<sup>3</sup> Not included in statistical analysis

<sup>4</sup> Effective s.e. for differences from white clover

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## Conclusions

The present results have important implications for the choice of alternative pasture legumes. The legumes used in the present study have all been suggested as having potential for the tussock grassland (Keoghan 1985). However, it is clear that problems **with both** germination and seedling survival have the potential to limit the success of some of the cultivars.

The most important features of the results are :-

1. Crown **vetch** is unlikely to have a place for oversowing in the tussock grasslands because germination limitations and slow seedling **growth** under low spring temperatures are likely to prevent establishment.
2. Monaro Caucasian clover appears to have similar germination and nodulation characteristics to white clover indicating **its** suitability for oversowing.
3. Further research is required to select **amore** suitable strain of rhizobia for **Treeline** Caucasian clover and birdsfoot trefoil to improve **nodulation** and hence establishment under oversowing.
4. Zigzag clover is **similar to white clover in germination** and nodulation at lower altitudes but germination appears to be restricted at higher elevations.

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