THE EFFECTS OF INOCULATION, PELLETING, RATE OF LIME AND TIME OF SOWING ON ESTABLISHMENT OF WHITE CLOVER

N. A. CULLEN

Director, Invermay Agricultural Research Centre

T.E. LUDECKE

Scientist, Ruakura Agricultural Research Centre

FOR SUCCESSFUL ESTABLISHMENT OF oversown clovers on virgin tussock grassland or bracken fern country, inoculation of the seed has proved beneficial (During et *al.*, 1962; Lowther, 1966).

In Australia, lime pelleting plus inoculating the seed has been shown to improve establishment, particularly on acid sites (Loneragan *et al.*, 1955), and in New Zealand the use of lime and other pellets is widely recommended (Hastings and Drake 1963; D.S.I.R. Report 1966).

Advantages claimed for this process are:

(1) Seed germination is enhanced.

(2) Pelleting protects the rhizobia when sowing on dry, uncultivated ground.

(3) Pelleting aids the survival of rhizobia on the seed and hence a delay in sowing is of less consequence than with normal inoculation only.

(4) Pelleted seed can be mixed with superphosphate without harmful effects.

(5) Pelleting can aid establishment on acid soil where the pH is marginal for nodulation.

In New Zealand, there has been little published work supporting these contentions although it has been estimated that 200,000 lb pelleted seed was sown in 1965 (D.S.I.R. Report 1966) and there is some evidence (Adams, 1964; Lowther, 1966) that pelleting has caused a depression in establishment.

TABLE 1: DETAILS OF TRIAL AREAS

Site	Rainfall Aug. 1965 to July 1966 (in.)	Approx. Altitude (ft)	Soil pH (O-1 in. depth)	Dominant Vegetation and Ground Cover
Traquair (Hindon)	25	1,400	4.9	Browntop and fescue tussock. Fairly dense cover,
Doon (Berwick)	30	1.800	4.4	Snowgrass and native herbs. Dense cover.
Brown (Berwick)	30	1,800	4.6	Burnt tussocks, herbs and much bare ground.
Moke Lake	32	1,500	5.5	Browntop. Dense cover.
Mt. Burke (Hawea)	22	1,400	5.8	Fescue tussock and herbs. Some. bare ground.
Thompson's Gorge (near Omakau)	23	2,300	5.8	Fescue tussock, herbs, native grasses. Dense cover.

Claims by Australian workers and reports by Hastings and Drake (1963) in New Zealand that rhizobia survival has been aided with pelleting have not been corroborated in trials at the Plant Chemistry Division, D.S.I.R. (Triennial Report 1964).

As lime pelleting gave results similar to 2 cwt of lime on acid soils in Australia (Loneragan *et* al., 1955) it was decided to investigate this. aspect on very acid soils in Otago.

Method and Material

Six trials were sown throughout the province in August, 1965, to obtain a wide coverage of soil and climatic conditions. Details of the sites are given in Table 1. The trials included four seed treatments :

(1) White clover seed not inoculated or pelleted. (The seed was treated with alcohol to destroy any rhizobia already present on the seed.)

(2) Seed inoculated but not pelleted.

(3) Seed inoculated and lime pelleted.

(4) Seed inoculated and pelleted with Gafsa* phosphate and dolomite.

Two times of sowing-August and February-and several rates of lime were also included. August was chosen as late winter and early spring are recommended

'A finely-ground North African rock phosphate.

TABLE 2: GERMINATION PERCENTAGES FOR AUGUST AND FEBRUARY SOWINGS
(Mean of four inoculation treatments)

Site	Sown Aug., 1965 %	Sown Feb., 1966 %
Traquair	40	35
Berwick (Doon)	32	28
Berwick (Brown)	36	41
Moke Lake	64	9
Mt. Burke	36	8
Thompson's Gorge	29	25

for oversowing (Lobb, 1958; During *et al.*, 1963) and the February sowing was included so that the effect of pelleting on rhizobia survival in hot, dry soil conditions could be investigated. Four rates of lime, nil, 5, 10 and 20 cwt were included in four trials, and two rates, nil and 20 cwt, in the other two trials.

The pelleted inoculated seed was supplied by A. Hastings, Plant Diseases Division, D.S.I.R., Auckland. The inoculated and surface sterilized seed was prepared at Invermay Agricultural Research Centre. The sowing at all sites was made within 14 days of the seed being treated.

All trials were sown using a single seed technique developed at Invermay Agricultural Research Centre (Cullen, 1965) in which 50 clover seed were sown in each plot at 3 in. spacing. This enabled an accurate assessment of germination and survival to be made. Seedlings were noted throughout the year and the vigour and height of each plant recorded.

Results

Weather conditions were generally favourable for establishment and rainfall was above average at most sites.

Germination counts were made 2 to 3 months after sowing and nodulation data were derived from counts of healthy plants present about six months after sowing. At that time clovers which had nodulated were vigorous, healthy and 1 to 2 in. in height while non-nodulated plants were stunted, yellow and usually less than ½ in. in height. Germination percentages for August and February sowings are shown in Table 2.

Main features of interest are the large differences between August and February sowings at the Moke Lake

TABLE 3: GERMINATION AND NODULATION DATA
(Total of six trials)

Treatment	Sown Aug., 1965		Sown Feb. 1966		Total	
	No. Germinated	% Nodulated	No. Germinated	% Nodulated	No. Germinated	% Nodulated
1. Not inoculated	956	17	612	12	1568	15
2. Inoculated	930	25	616	19	1546	23
3. Lime pelleted	947	14	509	7	1456	11
4. Gafsa/dolomite pelleted	966	16	592	8	1558	13

and Mt. Burke sites. The spring germination at Moke Lake amidst dense browntop was remarkably high and compared favourably with white clover germination on a well-prepared seedbed on cultivated ground. The reason for the low summer germination at Moke Lake is not clear but could be associated with the dense vegetative cover as rainfall and soil moisture were adequate.

Germination and nodulation data (mean of six trials) are given in Table 3. Neither lime nor Gafsa/dolomite pelleting resulted in a significant increase in germination at any of the six sites; rather, there was a tendency for a lower germination in the lime pelleted treatment especially when sown in February.

Results of the six individual trials are given in Table 4.

Most important features of the nodulation data are the marked depressions due to both lime and Gafsa/dolomite pellets at the Moke Lake and Mt. Burke sites. This effect was also evident in February sowings at the other sites. At Moke Lake the depression due to pelleting was extremely marked in the February sowing and few plants nodulated in these treatments,

Percentage nodulation for the three sites in South Otago at Traquair and Berwick was generally low for both August and February sown seed in all treatments. The reason for the low percentage nodulation is not known but it is suspected that an inhibitory substance could be present in the soil at these sites. At Moke Lake and Mt. Burke nodulation was fair in the inoculation only treatment although poor in the others, and it seemed unlikely that inhibitory factors were of any major consequence at these sites.

TABLE 4: GERMINATION AND NODULATION DATA FOR EACH TRIAL

Site	Rate of Lime Cwt.	Sown Aug., 1965								Sown Feb., 1966							
		Non-inoculated		Inoculated		Lime Pelleted		Gafsa/ Dolomite Pelleted		Non-inoculated		Inoculated		Lime Pelleted		Gafsa/ Dolomite Pelleted	
		No. Germ.	% Nod.	No. Germ.	% Nod.	No. Germ.	% Nod.	No. Germ.	% Nod.	No. Germ.	% Nod.	No. Germ.	% Nod.	No. Germ.	% Nod.	No. Germ.	% Nod.
Traquair	0	88	5	108	10	65	14	66	22	73	10	63	17	75	8	77	9
	20	84	19	80	19	80	22	77	28	77	17	72	14	57	2	66	5
		172	12	188	14	145	19	143	25	150	13	135	16	132	5	143	10
Berwick	0	64	2	77	4	65	3	59	0	59	12	58	12	54	2	55	4
(Doon)	20	51	8	79	8	53	8	59	7	58	24	61	41	46	4	63	8
		115	4	156	6	118	5	118	3	117	18	119	27	100	3	118	6
Berwick	0	29	0	31	0	45	0	35	0	35	0	42	10	33	0	37	0
(Brown)	5	39	8	37	11	34	6	43	12	40	17	41	lo	31	6	56	12
	10	43	14	33	18	32	12	40	5	34	12	45	4	35	9	47	15
	20	26	19	32	16	35	11	38	16	50	8	46	4	36	3	41	0
		137	10	133	11	146	7	156	8	159	9	174	7	135	4	181	8
Moke Lake	0	66	23	70	59	60	36	59	25	12	0	7	0	5	0	7	7
	5	65	46	68	31	62	16	71	31	10	0	7	29	6	0	7	0
	10	60	20	52	46	64	20	65	23	14	14	9	66	9	11	9	22
	20	64	36	61	52	62	24	77	16	4	0	10	70	13	15	10	0
		255	31	251	47	248	24	272	24	40	5	33	45	33	9	33	9
Mt. Burke	0	32	25	32	47	38	5	33	9	14	0	9	11	4	0	9	0
	5	28	7	29	48	36	8	35	0	7	14	14	21	7	0	15	7
	10	38	11	33	33	47	0	41	10	10	20	4	0	6	0	6	0
	20	39	28	32	47	49	14	40	20	5	0	12	33	7	14		0
		137	18	126	44	170	7	149	10	36	8	39	21	24	5	3:	3
Thompson's	0	32	6	20	15	35	14	28	7	22	9	35	31	13	0	15	0
Gorge	5	35	11	15	7	27	19	31	26	31	23	28	36	25	12	18	0
	10	37	19	20	10	31	13	30	13	29	0	19	21	26	23	24	8
	20	35	17	21	10	27	11	39	21	28	18	34	21	21	24	25	22
		140	14	76	11	120	14	128	17	110	13	116	28	85	16	82	12

EFFECT OF LIME

Lime aided nodulation at the Traquair and Berwick sites but had less effect at the three Central Otago sites. Here lime appeared to have little effect during the spring but aided nodulation with February sowings. At Berwick there appeared to be no advantage in applying more than 10 cwt lime despite the extremely low pH. Although lime improved the percentage of plants which nodulated there was no. noticeable improvement in clover vigour or height of the nodulated plants at any of the sites.

Discussion and Conclusions

In this series of six trials conducted on a wide range of soils throughout Otago, pelleting white clover seed with lime or Gafsa/dolomite in addition to inoculation failed to show any benefit. At two of the sites there was a very marked depression to both types of pellets and some evidence of a depression was noted at the other four sites.

Pelleting did not improve either germination or establishment : in most cases percentage nodulation was no better or even poorer than the non-inoculated seed. As there appeared to be a close relationship between nodulation in the pelleted treatments and the untreated seed it is suggested that any nodulation which occurred was largely from native rhizobia present in the soil. The greatly increased percentage nodulation in the inoculated only treatment, however, indicated that this treatment was of considerable value at several sites.

Lime aided nodulation particularly on the acid sites at Berwick and there was almost complete failure in its absence. In Australia, lime pelleting had aided establishment on acid soils and obviated the need for low rates of lime but at Berwick there was no response to lime pelleting and it was obvious that lime pelleting could not be used as a substitute for lime at this site.

August sowings were generally superior to February despite favourable growth conditions during the summer and autumn, and thus confirmed previous recommendations in regard to time of sowing.

Samples taken at the time of pelleting were subsequently tested by A. Hastings and shown to be effective although rhizobia survival was low after one month. Commercially

prepared pellets sown at Berwick about the same time, however, failed to give better results. No tests were done to determine the relative numbers of rhizobia on the seeds at the time of sowing.

As the percentage nodulation was low at all sites and even in the best treatment failed to exceed 50%, it is apparent that there is a need for further work to elicit the reason for the lack of nodulation. In Australia, clover inoculation problems have been encountered in a number of areas and nodulation failure in some cases attributed to microbial antagonism and to the effect of an antibiotic substance produced by fungi (Parker, 1962; Hely *et* al., 1957). Vincent (1959) found that a toxic substance was present in the seed of subterranean clover and that it diffused rapidly from the seed coat after moistening. Bowen (1961) isolated an antibiotic from the seed coat diffusate of subterranean clover and considered that poor survival with lime pelleting could be attributed to this cause. Brockwell (1962) investigating the effect of stickers found rhizobia survival after ten days to be much greater with gum arabic than with methyl cellulose. However, Parker and Oakley (1965) obtained significant depressions with lime pelleting of lucerne and serradella using gum arabic sticker as well as methyl cellulose.

This spring, a comprehensive series of trials including new pelleting materials, as well as various lime, fertilizer, and chemical treatments has been laid down by Invermay Agricultural Research Centre staff in an endeavour to clarify some of the problems.

In this paper, data have been presented for white clover only and it is stressed that any recommendations apply to this clover only. Lucerne was not included in this series but there is evidence to suggest that lime pelleting of lucerne seed can give excellent results.

With present knowledge it is recommended that white clover seed oversown on acid soils in Otago should be inoculated shortly before sowing but not pelleted. Present trial work with new pelleting materials, however, could affect this recommendation. In South Otago acid soils with a pH below 5.0, 10 cwt of lime should be sown, but lime is not considered necessary in Central Otago.

Depite superphosphate being considered highly toxic to rhizobia (Cass-Smith and Goss, 1958; Hallsworth, 1958),

T. E. Ludecke and J. A. Douglas (pers. comm.) in a trial at the Mt. Burke site found that inoculated white clover seed could be mixed with superphosphate for five minutes without affecting nodulation adversely and that lime pelleting was not superior to inoculation only. When the inoculated seed and the lime pelleted plus inoculated seed were mixed with superphosphate for one day clover nodulation in both treatments was greatly reduced. Any contact of rhizobia with an acid fertilizer such as superphosphate nevertheless is best avoided and separate sowing of seed using a spreading device to obtain improved seed distribution is recommended for aerial sowing.

Acknowledgements

The writers would like to acknowledge the help of C. Feyter, I. R. McDonald and others for their valuable assistance in sowing and recording the trials and for their reading and recording of the rainfall and other meteorological data. The assistance of A. Hastings in the preparation and testing of the pelleted material is also gratefully acknowledged.

REFERENCES

Adams, A. F. R., 1964: *Proc. 26th Conf. N.Z. Grassl. Ass.,* 115-22.
Bowen, G. D., 1961: *Plant and Soil, 15*: 155–65.
Brockwell, J., 1962: *Aust. J. agric. Res., 13: 638-49.*
Cass-Smith, W. P.; Goss 0. M., 1958: *J. Agric. W. Aust.,* 119-21.
Cullen, *N.* A., 1965: N.Z. *agric. Sci., 1*:25–7.
During, C.; Cullen N. A.; Bennett, G. M., 1962: N.Z. *J. agric. Res., 5*:278–*93.*
During, C.; Cullen, N. A.; Mountier, N. S., 1963: N.Z. *J. agric. Res.,* 6:416-31.
Hallsworth, E. *G.,* 1958: The *Nufrition of Legumes.* Proc. Univ. of Nottingham. Butterworths, London, p. 183.
Hastings, A.; Drake, A. D., 1963: N.Z. *J. Agric., 106*: 463–8.
Hely, F. W.; Bergersen, F. J.; Brockwell, J.. 1957: *Aust. J. agric. Res., 8: 24-44.*
Lobb, W. R., 1958: *Proc. 20th Conf. N.Z. Grassl. Ass.: 50-64*
Loneragan, J. F.; Meyer, D.; Fawcett, R. G.; Anderson, A. J., 1955: *J. Aust. Inst. agric. Sci., 21*: 264–5.
Lowther, W. L., 1966: M. Agr. Sc. thesis, Lincoln College, Canterbury.
N.Z. Dept. Sci. Indust. Res., 1966: *Ann. Rep. for Year Ended 31 March* 1966: p. 16.
Parker, *C.* A., *1962: J. Agric. W. Aust., 3.*
Parker, C. A., Oakley R. E., 1965: *Aust. J. expt. Agric. & Anim. Hus.,* 5: 144–6.
Plant Chemistry Division, D.S.I.R., 1964: *Triennial Report 1961–64.*
Vincent J. M., 1959: *The Nutrition of Legumes.* Proc. Univ. of Nottingham. Butterworths, London, 108.

DISCUSSION

Were the same cultures of rhizobia used for both seed treatments? Were the bacterial counts on the coated and uncoated seed at sowing similar?

The same culture was used. Rhizobia counts were not taken at sowing.

In the time interval between preparation of inoculated coated seed and its sowing in the field, culture contaminants could have had an effect on the viability of rhizobia on that treatment. With inoculation of the uncoated seed just prior to sowing, there would not be the same effect. Would this have affected your subsequent comparison?

The reason for the poor results with pelleting is not known. Culture contaminants could be important.

You have obtained poor results in attempting to establish clovers in areas such as at Berwick. Your method of inoculating seed prior to sowing is likely to be more precise than that of the average farmer. Therefore, would you agree that it is as yet inadvisable for the farmer in such areas to attempt to establish clover?

Despite establishment difficulties, successful establishment is possible. We recommend inoculating the seed shortly before sowing and increasing the rate of clover sown to offset the low percentage of nodulating plants.

Differences in the ease with which successful nodulation of lucerne and white clover may be obtained suggest that different mechanisms may be involved. Is this the case, and if so, what is known about these mechanisms?

White clover and lucerne appear to behave in a different manner. They have a different pH requirement, for one thing. Australian work indicates that an antibiotic is present in the seed coat of legumes which could affect the rhizobia. This substance is more pronounced in subterranean clover than in lucerne.

In areas such as Galatea (Rolorua), lime pelleting of seed did not aid establishment of legumes and successful results were obtained only where the seed was sown with large quantities of lime. What was the effect of broadcasting lime in your trials and would better results have been obtained if a massive quantity of lime was placed in proximity with the seed?

This aspect is being studied on this season's trial.

COMMENT (Dr C. S. W. REID): In soil seeding tussock grassland in the Hindon area with legumes, application of lime six weeks prior to sowing had no effect on establishment, but where applied twelve to eighteen months previously, results were better.

COMMENT (Professor T. W. **WALKER**) : Positive and negative results with lime pelleting have 'been obtained at various as well as at the same locations. The reasons for these variable results have not yet been elucidated and it is apparent that until the concerted effort of several scientific disciplines is directed at this problem, little real progress is likely. to be achieved.