RED CLOVER (*TRIFOLIUM PRATENSE* L.) SEED QUALITY

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ABSTRACT

Seed viability in red clover (*Trifolium pratense* L.) is usually high once hard seed has been broken through scarification, germination values of around 90 % are common. However, when seed lots of similar high germination are sown, field emergence may vary considerably among lots. We therefore investigated whether seed vigour differences exist among seed lots of *T. pratense* cv. Grasslands Pawera.

Six seed lots with germinations ranging from 88 - 92 % were subjected to accelerated ageing (AA), controlled deterioration (CD) and conductivity vigour tests, and differing responses were recorded; for example seed lot germination after CD (16 % seed moisture content, 24 hours at 45 °C) ranged from 48 - 78 %. Similar differences in seed lot response were recorded in the other tests used, and so 'high' and 'low' vigour lots could be differentiated.

Vigour test results and standard germination were correlated with field emergence following three spring and three autumn sowings at the same site. Stress vigour tests (CD and AA) were more closely related to field emergence than standard germination, and were good predictors of plant population. Conductivity vigour tests were not significantly correlated with field emergence.

Low vigour seed lots produced smaller, slower growing seedlings following autumn field sowing. The implications for establishment and dry matter production are being further investigated.

***Additional keywords:** seed vigour, field emergence, establishment, vigour testing.*

INTRODUCTION

Seed quality has been defined as a collection of seed properties which are considered to be of importance for the value of seed for sowing purposes (Esbo, 1980), and traditionally, information about three of these properties *viz* cultivar purity, analytical purity and germination, has been required by the seed trade and farmers. For New Zealand red clover seed lots, cultivar purity is assured through the seed certification scheme and analytical purity standards are generally met through effective production and processing techniques (Scott & Hampton, 1985). Germination is complicated by the presence of hard seeds; that is, seeds which fail to imbibe water within the prescribed test period. For example, of the 550 red clover seed lots tested in 1983 -1985, only 12 % had a germination of > 90 %, and 63 % had a germination of > 80 % (Hampton *et al.*, 1987). In 1984, 15 % of the seed lots had a hard seed content of > 10 % (Scott & Hampton, 1985). Once hard seed has been broken for example by scarification (Hare & Rolston, 1985) a germination of around 90 % can be expected (Hampton, unpublished data).

Germination testing is designed to provide information about the planting value of a seed lot, and remains the principle and internationally accepted criterion for seed viability. However, field performance differences may occur between seed lots which the germination test indicates are of similar quality (e.g. Table 1). The reason for these differences is ascribed to seed vigour, a further component of seed quality.

Seed vigour refers to those properties of the seed which influence the ability to perform under a wide range of environmental conditions. Lack of seed vigour may result in slow or uneven field emergence, or failure to establish as well as laboratory test results otherwise indicate (Scott & Hampton, 1985). Vigour differences exist and are important agronomically in many large seeded legumes (e.g. Hampton & Scott, 1982). In herbage legumes, information is as yet limited. McKersie *et al.* (1981) and Charlton (1989) linked seed lot performance differences to seed size in *Lotus corniculatus* L., and of *L. pedunculatus* Cav., but the relationship was not consistent. Wang (1989) detected vigour differences between seed lots of *Trifolium repens*

Table 1: Field emergence of four Grasslands Pawera red clover seed lots which germination data indicate are of similar quality (Wang, 1989)

Seed Lot		1	2	3	4	LSD (P < 0.05)
Germination (%)		90	90	90	90	NS
Field emergence (%)						
Lot	1	76	56	78	80	10.6
Lot	2	75	59	78	79	11.3

L. and of *Medicago sativa* L. In 1988 an experiment was designed to determine whether vigour differences exist between seed lots of *T. pratense* cv. Grasslands Pawera, and what effects they may have on field performance.

MATERIALS AND METHODS

Seed lots: Six seed lots of certified first generation Grasslands Pawera red clover were supplied by Challenge Seeds Ltd., Christchurch. These lots were selected because they had been harvested in 1988, were all from the same certification class, and all had germinations of around 90 %.

Quality assessments: Sampling and testing procedures followed those laid down by international agreement (ISTA, 1985). Four replicates of 50 seeds per seed lot were germinated using the top of paper method at 20 °C, and at the 10 day final count, seedlings were classified as normal, abnormal, hard or dead as prescribed in the rules (ISTA, 1985). Seed moisture content was determined by drying two replicates of 50 weighed seeds per seed lot at 130 °C for 1 h, cooling in a desiccator for 30 m, re-weighing and calculating on a wet weight basis. Thousand seed weight was determined by meaning the weight of four replicates of 100 seeds per seed lot and multiplying by ten.

Vigour testing: Three vigour tests, conductivity (ISTA, 1987), accelerated ageing (AOSA, 1983) and controlled deterioration (ISTA, 1987) were used for each seed lot.

1) *Conductivity* - four replicates of 0.5 g seed from each seed lot were each placed in 100 ml distilled water in a 125 ml flask, covered and held at 20 °C for 24 h. The flask contents were then stirred and the conductivity of the soak water measured using a Radiometer CDM-83 conductivity meter. Two measurements were recorded per flask and the mean result expressed as microsiemens per gram of seed.

2) *Accelerated ageing* - four replicates of 0.5 g seed per seed lot were tied into muslin cloth bags which were then placed on a metal grid inside a sealable "Agee" 85 mm diameter preserving jar. The jar contained 100 ml water and the seed was supported 30 mm above the water surface. Each jar contained one replicate of each of the six seed lots. The jars were then sealed and placed at 40 °C for 24 h for one jar set, and 48 h for a duplicate set. After ageing, seed was removed and given a standard germination test.

3) *Controlled deterioration* - four replicates of 0.5 g seed per seed lot were each placed in aluminum foil bags. For each seed lot, seed moisture content (SMC) was then adjusted to 16 % by the addition of water to the bag. The required amount of water was calculated from the formula -

$$W_2 = \frac{100 - A}{100 - B} \times W_1$$

where A = initial SMC, W_1 - W_2 = water to be added. After adding water, each bag was heat sealed, placed at 10 °C for 24 h to allow slow and even inhibition, and finally placed at 45 °C for 24 h. After this ageing, seed was removed and given a standard germination test.

Field emergence: Four replicates of 50 seeds per seed lot were hand sown on 23 September, 18 October, 13 November 1988, and 21 March, 5 April and 20 April 1989 into a sandy loam soil at Massey University.

A randomized complete block design was used. Seeds were sown at 10 mm depth, with 30 mm between seeds within the row and 150 mm between rows. Emergence counts were begun on the day the first seedling was visible and continued at two day intervals until no further increases in seedling numbers were obtained. Seedling length and dry weight were recorded 8 weeks after spring sowings and 5 weeks after autumn sowings by digging up 10 seedlings per row at random, washing soil from the roots, measuring root and shoot length, and obtaining dry weights after seedlings had been placed at 65 °C for four days.

RESULTS

Seed quality: Germination testing confirmed that the six seed lots chosen all had germinations of around 90 %, ranging from a low of 88 % for seed lot 1 to high of 92 % for seed lot 5 (Table 2). There were no significant differences in the percentage of normal or abnormal seedlings between seed lots, and hard seed levels were low. The seed lots did differ in thousand seed weight and seed moisture content (Table 2).

Seed vigour: Vigour testing produced significant differences among seed lots (Table 2), in that lots 1 and 3 germinated poorly after the stress tests (AA and CD), whereas the germination of lots 2, 4, 5 and 6 did not decrease as markedly. Increasing the stress time from 2 to 3 days in the AA test further separated the seeds lots (Table 2). Conductivity results did not exactly parallel the stress test results, because although seed lots 1 and 3 had high conductivities, they did not differ from that of seed lot 5 (Table 2).

Field emergence: Significant differences in field emergence among seed lots were recorded at five of the six sowings (Table 3), and the low vigour seed lots (1 and 3) always had the lowest emergence. The emergence of the other four seed lots did not differ at 4 of the 6 sowings, although the ranking order was not always consistent.

Germination data were not significantly correlated with field emergence, with the exception of the last spring sowing (Table 4). All three vigour tests were more strongly related to field emergence than was germination, again with the exception of the 13 November sowing. Of the vigour tests, controlled deterioration provided the most consistent relationship over all sowings.

At all three spring sowings, there were no differences in seedling dry weight between seed lots. However in autumn, the seedling dry weight of lots 1 and 3 was significantly reduced. Table 5 presents data for seed lots 3 (low vigour) and 4 (high vigour).

DISCUSSION

This study has confirmed that vigour differences exist among seed lots of red clover, cv. Grasslands

Table 2: Grasslands Pawera seed lot quality: germination thousand seed weight, seed moisture content and vigour.

	Seed Lot					
	1	2	3	4	5	6
Standard germination test						
Normal seedlings (%)	88*	90	90	90	92	90
Abnormal seedlings (%)	9	7	5	6	6	7
Hard seed (%)	2ab	3ab	1b	4a	1b	2ab
Thousand seed weight (g)	4.1a	3.2d	3.7b	3.8b	3.6c	3.9b
Seed moisture (%)	11.9b	11.8b	12.4a	10.9c	12.1a	11.3b
Vigour tests						
Conductivity (μS/cm/g)	99.9b	95.9b	105.7a	91.4c	103.3ab	97.8ab
CD+	50b	75a	48b	74a	78a	78a
AA 2 db	59b	82a	50b	76a	74a	73a
AA 3 db	23c	63a	7c	42b	60a	39b

* within rows any number followed by a different letter is significantly different at $P < 0.05$; + % normal seedlings.

Table 3: Field emergence and seed lot ranking.

Sowing date	Field emergence % range	mean	Seed lot ranking*					
23 September	60 - 83	75	4	6	2	5	3	1
18 October	29 - 63	51	4a	6ab	2ab	5abc	3bc	1c
13 November	49 - 71	61	5	2	6	4	3	1
21 March	46 - 80	66	6a	4a	2a	5a	1b	3b
5 April	59 - 79	71	6a	5a	4a	2a	1a	3b
20 April	56 - 89	65	4a	6ab	5b	3b	3c	1c

* lots followed by a different letter are significantly different (P < 0.05)

Table 4: Correlation between field emergence and laboratory quality assessments.

Sowing date	Standard germination	Conductivity	Controlled deterioration	Accelerated ageing (3 d)
23 September	0.62	-0.66	-0.96**	0.82*
18 October	0.39	-0.76	0.74	0.43
13 November	0.93**	-0.17	0.83*	0.72
21 March	0.43	-0.74	0.94**	0.72
5 April	0.41	-0.61	0.94**	0.83*
20 April	0.57	-0.66	0.89*	0.59

* P < 0.05, ** P < 0.01.

Table 5: Effect of seed vigour on seedling dry weight for autumn sowings.

Sowing date	Seedling dry weight (mg)		
	"Low vigour"[a]	"High vigour"[b]	LSD (P < 0.05)
21 March	49.1	64.0	10.6
5 April	24.5	34.0	7.8
20 April	21.3	29.8	7.4

[a] seed lot 3, [b] seed lot 4.

Pawera which did not differ in laboratory germination and were of the same chronological age. The results are similar to those previously reported for large seeded legumes such as garden pea (Hampton & Scott, 1982), soybean (TeKrony & Egli, 1977) and field bean (Hegarty, 1977), and more recently for other herbage legumes (Wang, 1989).

Many reports have linked seed physical properties to seed vigour. For example Charlton (1989) reported a strong correlation between seed size and germination rate in *Lotus pedunculatus*, but also noted that one seed

Table 6: Field environmental conditions[a]

Sowing date	10 cm soil temperature °C Day 0[b]	Day 10	Soil moisture content at at sowing %	Rainfall (mm) during emergence	Days to final emergence
23 September	12.2	12.7	26.3	45.6	14
18 October	13.6	13.8	24.8	50.3	20
13 November	12.2	14.4	18.5	47.9	18
21 March	12.3	16.2	21.0	18.0	14
5 April	12.5	11.6	15.0	18.6	20
20 April	12.8	11.8	14.9	67.5	15

[a] recorded on site, [b] day of sowing.

lot was exceptionally slow to germinate despite having the largest seeds. Such inconsistencies have been reported in other herbage species (Scott & Hampton, 1985), and were demonstrated once more with Grasslands Pawera, when seed lot 2 (TSW = 3.2 g) was of higher vigour than seed lot 1 (TSW = 4.1 g). However it is now generally accepted that the causes of vigour differences in many species are physiological (Powell, 1988), rather than directly physical, although the latter may influence the former. Maximum seed quality occurs at physiological maturity, after which vigour and viability decline both during ageing on the plant and during storage; vigour may decline faster than viability. Seed ageing involves the process of deterioration - biochemical changes which follow a generalised sequence and involve changes in solute leakage, enzyme activity, respiration and ATP content, protein and DNA synthesis, the chemical content of the seed and genetic changes. All of these deteriorative metabolic changes observed in aged seeds may be the consequence of initial membrane deterioration (Powell, 1988), and the integrity of cell membranes, determined by deteriorative biochemical changes and/or physical disruption, can therefore be considered to be a fundamental cause of differences in seed vigour. The factors influencing deteriorative biochemical changes have been recently reviewed (Powell, 1988), but what is not yet known is whether the evidence accumulated for large seeded legumes is also directly applicable to small seeded legumes. Physical disruption can occur during seed maturation, harvesting and processing, but may not necessarily be severe enough to be visible externally. However, evidence of some physical damage is common in New Zealand red clover, particularly when seed has been harvested at unsuitable moisture contents or incorrect scarification techniques have been used (J.E. Miller, pers.comm.) - such damage in its severest form show up as dead or abnormal seedlings in a germination test. More subtle, but yet very important damage is unlikely to be detected in the germination test. The effects of seed production and processing practices on red clover seed quality require further investigation.

The three vigour test methods used distinguished "low" from "high" vigour seed lots, and with the exception of one sowing, provided a better indication of actual field emergence than the standard germination test. The conductivity and accelerated ageing tests have been significantly correlated with field emergence in large seeded legumes (Powell, 1988), while the controlled deterioration test provided a better indicator of field emergence than the germination test for a number of small seeded vegetable species (Powell & Matthews, 1981). The superiority of the controlled deterioration test in predicting field emergence of Grasslands Pawera red clover requires confirmation with other seed lots and at more sites, but appears to be associated with reduced variation and consistency of results as compared to those for accelerated ageing (Wang, 1989). The principles of the tests and advantages and disadvantages of each method have been more fully discussed by Wang (1989).

Vigour differences among seed lots usually become apparent when the seed lots are subjected to environmental stress, and commonly, temperature is an important factor, particularly with autumn sowings (Hampton *et al.*, 1987). However, field environmental data recorded (Table 6) do not suggest an obvious link, as the 1989 autumn was relatively mild, and days to final emergence did not differ markedly between the spring and autumn sowings. Soil physical properties (structure, moisture content) may impose limitations on seed establishment,and it is possible that lower soil moisture for the April sowings (Table 6) may have influenced seed lot response. This requires clarification.

It could be argued that the establishment of vigour differences among Grasslands Pawera seed lots, and the indication that significant differences in seedling performance exist with autumn sowings is on no practical significance, because farmers usually use sowing rates far higher than are actually needed. However, Thom *et al* (1985) noted that the tendency for high seed rates to be used in practice probably reflects farmers lack of confidence in the ability of species to establish under their particular farm circumstance. Lancashire (1985) noted that around 80 % of farmers sow pasture seeds in autumn, and Hampton *et al.* (1987) reported the negative influence of low autumn temperatures on germination rate. If this is further complicated by poor seed vigour, the ability to establish, produce dry matter and survive, particularly in a competitive multispecies sward becomes extremely important. Further work is required to confirm this, not only for Grasslands Pawera red clover, but also for other red clover cultivars and herbage legume species.

CONCLUSIONS

1. Vigour differences exist among seed lots of Grassland Pawera red clover with similar germinations.
2. Both the controlled deterioration and accelerated ageing vigour tests (stress tests) differentiated "high" and "low" vigour lots.
3. Field emergence and seedling performance differed among lots at five out of six sowings at the same site.
4. The stress tests (particularly controlled deterioration) proved better indicators of field emergence than the standard germination test.
5. The effects of seed lot vigour on establishment and dry matter production require further investigation.

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