Quality Factors in White Clover Seed Production[1]

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ABSTRACT

Seed quality refers to a number of seed properties which have varying degrees of practical importance for agriculture. To conform with both OECD and domestic seed certification schemes analytical purity, germination and cultivar purity standards must all be met.

The presence of excess or prohibited weed species or contamination with other crop species are common reasons for the downgrading or rejection from certification of seedlots of white clover *(Trifolium repens L.)*. Seeds of *Sherardia arvensis* and *Rumex acetosella* are often damaged during harvesting and become particularly difficult to remove from the seedlot during seed cleaning. Germination is rarely a quality problem in white clover, although germination rate may be influenced by seed weight. Management practices designed to increase seed weight usually reduce seed yield.

There is a world wide problem of genetic contamination in white clover seedlots. Cultivar purity testing using spaced plants on seedlots from Europe and New Zealand has shown that many have significant differences in plant growth and leaf size, both between lots of the same cultivar, and also in comparison with the breeders standard seedlot. The NZ experience suggests that much of this contamination results from buried seed.

Surveys of arable soils in Denmark, UK and NZ have shown buried seed levels ranging from 0-18,000 seeds m^{-2} (0-128 kg ha^{-1}). Most of this originates from losses during harvest which may be from 10-40% of the crop yield. No successful method of reducing hard seed returned to the soil, or of promoting uniform germination of hard seed in the soil, has yet been devised. However, the NZ seed industry has recently developed production technology which will allow it to change cultivars and produce seed which meets all OECD requirements.

Additional index words: Trifolium repens; **seed quality; analytical purity; germination; seed weight; cultivar purity; cultivars; seed production; contamination; hard seed; buried seed.**

INTRODUCTION

Caradus (1986) summarized information about the origin, breeding and characteristics of 232 white clover (*Trifolium repens* L.) cultivars. These cultivars originated in 27 countries, with the number of cultivars per country ranging from one for Brazil, Chile, China, Israel and South Africa to 31 for the UK. While much has been published about their agronomic potential (Caradus, 1986), for most, relatively little is known about their seed yielding capabilities, and even less about the quality of the seed produced, particularly cultivar purity. In this paper we review the major quality factors involved with white clover seed production.

Analytical Purity

To conform with both domestic and OECD seed certification schemes, analytical purity standards must be met. Failure to meet the required standards for pure seed, other seed and inert matter results in downgrading to a lower certification class or rejection from certification. For example 14% of New Zealand's white clover seedlots from the 1985 harvest were downgraded or rejected from certification during purity testing, 7% because of the presence of excess or prohibited weed species, and 7% because of contamination with suckling clover (*Trifolium dubium*) (Young and Hampton, 1987).

Weed species such as *Trifolium glomeratum* (clustered clover), *Chenopodium album* (fathen), *Stellaria media* (chickweed), *Rumex acetosella* (sheep's sorrel), and *Sherardia arvensis* (field madder) are difficult to remove from white clover seedlots (Hartley, 1969). These species were common in NZ seedlots surveyed in 1968 (Dingwall, 1969) and were still major reasons for downgrading or rejecting seedlots in 1984 (Scott and Hampton, 1985). With the exception of *T. glomeratum* they also commonly occur in seed of English, Danish, Dutch and Swedish origin (Gooch, 1963; Holm, 1983). Seaton (1975) also reported that *Plantago major* and *Polygonum*

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aviculare occurred frequently in white clover seedlots.

Seeds of field madder and sheep's sorrel are often damaged during harvesting (Hartley, 1980), so that for the former the crown of awns has been removed while for the latter the rough enclosing perianth layer has been removed (Young and Hampton, 1987), making them similar in size to white clover and thus difficult to remove during machine dressing without substantial losses of white clover seed.

Germination

The germination of herbage legumes is complicated by the presence of hard seed (Scott and Hampton 1985), although for NZ white clover seedlots, hard seed content is rarely over 10%. A recent survey showed that for 1983-85, 96% of Grasslands Huia white clover seedlots germinated over 80%, and 73% of seedlots germinated over 90% (Hampton et al., 1987). These data are similar to those previously reported by Johnston and Miller (1960), and Foy (1926), and confirm that germination is rarely a quality problem in white clover, provided that seed is mature at harvest (Hyde, 1950).

Seed Weight

Germination rate and seedling vigor (the rate of extension of both root and shoot) are important factors in the establishment of herbage legumes (Hampton et al., 1987). Rapid early growth rates are partially dependent on seed reserves, and thus seed weight and seedling growth are often highly correlated (Scott and Hampton, 1985). Evans (1973) found that plants of red clover (*T. pratense*) cv Grasslands Hamua grown from low weight seeds were smaller and had shorter root lengths and slower root elongation rates than plants from medium and high weight seed.

Increasing seed weight in white clover cultivars may be desirable for improving plant establishment, but is also a factor in increasing seed yields (Davies, 1981). While this may be achieved through plant breeding (Lorenzetti, 1981), any management practices designed to enhance seed weight tend to reduce seed yield potential (Clifford, 1986b; 1987), and there appears little managerial scope to modify seed weight as the plant tends to maximize seed numbers per inflorescence taken through to maturity at the expense of seed filling (Clifford, 1986a). Assimilate shortages resulting from factors such as soil moisture deficits (Clifford, 1986a), sulphur deficiency (Clifford and White, 1986) or disease (Barnett and Gibson, 1977) significantly reduce seed weight. High seed weights can be produced with early closing, high soil moisture and low inflorescence populations, but always at the expense of yield. For example, a 17% increase in seed weight halved the associated seed yield (Clifford, 1979, 1986b).

Cultivar Purity

Maintenance of cultivar purity is the major reason for the existence of seed certification schemes (Anon, 1986), and participation in the OECD herbage and oil seed certification scheme requires that rules must be adhered to and standards met (OECD, 1977).

Seed multiplied outside the country of origin. Under the OECD scheme, seed may be multiplied outside the country of origin of a cultivar (OECD, 1977) and the designated certifying authority in the country of origin of the cultivar must be satisfied that the cultivar is likely to remain true to its description under the conditions proposed. Horne (1966) in reviewing the first seven years of the OECD herbage scheme noted that seed of 45 European cultivars had been multiplied in North America, and that no significant population shifts had occurred.

Population shifts during seed multiplication in different environments have been reported for white clover (Bula et al., 1964; Kelly and Boyd, 1966). A differential response of individual plants within a cultivar to daylength and temperature has been suggested as a possible source of such shifts since these environmental factors are known to influence both vegetative (Eagles and Othman, 1986) and reproductive mechanisms of the plants (Simon et al., 1974).

However, Bula et al. (1964) concluded that "differences amongst locations were not great enough to unquestionably conclude that progenies produced outside the region of origin differed from those produced within the region of origin", and Kelly and Boyd (1966) found no shift in leaf size and stolon thickness in their comparison of seedlots produced in UK, USA, Canada and Finland. Simon et al. (1974) found that plant characteristics and forage yield of West German white clover experimental composites were retained when seed was multiplied for two generations at three locations in the USA.

Limited preliminary data for seedlots of cultivars of European origin produced in NZ (Table 1) also suggested that parent and progeny may not differ significantly. In this trial there were no differences between the standard and NZ grown seedlots for any of the botanical characters recorded (J.G. Hampton and J.E. Miller, unpub data). Further data on more seedlots are being recorded.

Seed multiplied within the country of origin. Simon et al. (1974) compared white clover seedlots grown in West Germany and in USA with the original source material, and found that a genetic shift occurred during seed multiplication in W. Germany, the country of origin. Lancashire et al. (1985) also reported marked variations

Table 1. Comparison of leaf size[a] for standard and New Zealand grown seedlots of white clover cv. Donna and Aran.

Seedlot	cv. Donna Standard	cv. Donna NZ grown	cv. Aran Standard	cv. Aran NZ grown
Mean leaf size[b]	20.1	21.1.	20.6	19.9
Range	15.3-21.8	15.9-22.5	17.1-23.1	18.5-23.3
SD	1.19	1.06	1.54	1.34
CV (%)	5.9	5.0	7.4	6.7

[a] Third leaf from apex of a new shoot (Hawkins 1959), scored using the method of Williams et al. (1964)
[b] Mean of 30 plants

Table 2. Effect of region of production on growth score of white clover cv Grasslands Pitau, and buried seed load (after Lancashire et al. 1985).

Region of production	Growth Score[a] Mean[b,c]	Growth Score[a] Range	Seedlots significantly different from breeders standard	Buried seed count (mean)	
			(%)	(No. m^{-2})	(kg ha^{-1})
Marlborough	91	79-101	12	260	2.0
Ashburton	79	57-96	50	1390	10.0
Oamaru	73	62-77	89	1060	7.5

[a] See Lancashire et al. (1985)
[b] Grasslands Pitau breeders seedlot score = 100
[c] LSD $P<0.05$ = 19

Table 3. Range of growth scores and leaf sizes for some white clover cultivars (Lancashire et al. 1985)

Cultivar	Growth scores	Leaf sizes
Aran	50.6 - 67.8[a]	20.0 - 21.8[b]
Blanca	38.6 - 53.8[a]	17.8 - 19.4[b]
Menna	-----	16.9 - 18.5[b]
Milkanova	36.0 - 52.0[a]	17.5 - 19.6[b]
Sabeda	27.4 - 41.8[a]	17.3 - 19.2[b]
S184	23.6 - 41.0[a]	14.3 - 16.6[b]
G. Huia	42.9 - 52.8 NS	17.7 - 18.8 NS

[a] LSD 5% = 14.3
[b] LSD 5% = 1.46

in growth and morphological characteristics of seedlots of 'Grasslands Pitau' white clover grown in different regions of NZ (Table 2). Seedlots grown in Ashburton and Oamaru tended to have winter growth scores, leaf size and picric acid test scores for cyanogenesis (Doak, 1933) approaching those of Grasslands Huia white clover (Lancashire et al., 1985), which has smaller leaves than Grasslands Pitau (Caradus, 1986).

Buried seed counts for these southern areas (Table 2) suggest a probable answer to the source of contamination, as there was no relationship between certification class of the harvested crop, season and year of sowing and class of seed sown which would have confounded the overall regional effect (Lancashire et al., 1985).

Another possible factor in explaining the regional differences found is that natural selection of genotypes may occur if a cultivar is grown in areas and/or under management to which it is not adapted (Dovrat and Waldman, 1966). The production of Grasslands Pitau breeders seedlots which have proved true to type, in a cool environment free of buried seed suggests that genetic shift was not a factor in this case (Lancashire et al., 1985).

The production of pre-basic and basic seedlots of Grasslands Pitau white clover in these southern areas was stopped in 1984, and the growth score and leaf size of 10 basic and first generation Grasslands Pitau seedlots tested in 1986 did not differ significantly from the breeders standard (Hampton and Miller, unpub data).

Current seedlot cultivar purity. Certified white clover seedlots are grown under OECD seed certification rules, yet it appears there is a world-wide problem of genetic contamination (Lancashire et al., 1985).

(1) **New Zealand bred cultivars** NZ exports around 4,000 mt of white clover seed annually (Johnson and Hampton, 1987), 98% of which is Grasslands Huia. This supplies over 80% of the seed used in UK and many other European countries (Davies, 1981). Lancashire et al. (1985) considered that Grasslands Huia seedlots were fairly uniform, as differences in growth score and leaf size between 10 seedlots purchased through normal commercial outlets in the UK were not significant (Table 3). However, Hampton and Miller (unpub data) found in spaced plant evaluations that some certified seedlots differed from the breeders standard seedlot because of reduced growth score, and reduced leaf size. However the number of seedlots (24) in this study was too small to allow any definite conclusions to be drawn. Growth score data collection was complicated by the effects of stem nematode (Williams, 1972), particularly on basic seedlots (K.A. Young, pers comm). The influence of stem nematode infection on plot testing data requires further investigation.

(2) **Other cultivars.** Lancashire et al. (1985) reported that 35-40% of cultivars bred outside NZ showed significant differences in growth and leaf size between seedlots of the same cultivar (eg Table 3). This variation has been confirmed in a further study in 1985 (J.E. Miller, unpub data) which showed that breeders standard seed samples of seven cultivars of European origin contained significantly ($P<0.01$) more within-cultivar variation than four NZ bred cultivars for such characters as leaf size, leaf width, leaf:stolon ratio and stolon thickness.

Further data on within-cultivar differences for all cultivars from which certified seed is produced in NZ are being accumulated. However, a recent post-control plot test finding illustrates an important point. Basic seed of a European bred cultivar was imported and sown in NZ to produce first generation seed. The NZ produced seedlot had 14% of plants with leaf size greater than three times the standard deviation of mean leaf size for the breeders' standard (Hawkins et al., 1964). These plants were larger leaved than the standard,and also significantly larger than the leaf size for any NZ bred cultivar. The contamination could not therefore have arisen from buried seed, and was subsequently proved to have already been present in the imported basic seedlot. The complicated process of producing white clover seed requires that seed for further multiplication must meet all cultivar, as well as analytical, purity requirements.

The problem of variation in certified white clover seedlots is world wide. The NZ experience suggests that contamination from buried seed is probably the major reason for this. What then can be done about buried seed?

Buried Seed

Buried hard seed of white clover poses the major threat to maintaining white clover cultivar purity as required by OECD rules for certified seed production. Surveys of arable soils in Denmark, UK and NZ have shown buried seed levels ranging from 0-18,000 seeds m^{-2} (0-128 kg ha^{-1}), depending on site and previous cropping history (Milton, 1943, 1948; Hyde and Suckling, 1953; Jensen, 1969; Lancashire et al., 1985). Jensen (1960) reported finding viable buried white clover seed from 16/57 Danish fields surveyed, with populations ranging from 1-5,000 seeds m^{-2}, while in a more recent survey, Lancashire et al. (1985) found mean buried seed levels of 200-1000 m^{-2} (2-10 kg ha^{-1}) from arable soils in different districts of NZ. Buried seed levels in grassland soils are often greater than those recorded for arable soils (Suckling and Charlton, 1978) as soil disturbance encourages germination (Popay et al., 1983).

Hyde (1950) showed that over 90% of freshly ripened white clover seed is hard, i.e. viable but with a water impermeable seed coat which creates an innate dormancy (Rolston, 1978). While mechanical harvesting and cleaning usually results in sufficient physical force on the seed to reduce the hard seed content of a seed lot to acceptable levels (Scott and Hampton, 1985), seed losses before and during harvesting can return large quantities of hard seed to the soil (Clifford and McCartin, 1985).

Buried white clover seeds can remain viable for many years. The longest authenticated record for viability of buried white clover seeds is 30 years (Suckling and Charlton, 1978). Lancashire et al. (1985) reported that 22-25% of plants grown from scarified samples of buried seed collected in 1984 had no leaf mark, the characteristics of cv S100 NoMark, last grown for seed production in that field in 1970/71.

Experiments in the UK (Lewis, 1958; Roberts and Boddrell, 1985) suggest that most viable white clover seed in the 0-10 cm zone has germinated within 4-5 years. However, the seedlot used by Lewis had a hard seed content of only 8%. In NZ white clover is frequently sown and harvested every third year, so that for many arable soils, buried seed levels may not greatly diminish.

Reducing Buried Seed Levels

(1) Crop management. To minimize the hard seed content of seed returned to the soil, crop management should promote the shortest flowering span consistent with a high harvestable seed yield potential (Clifford et al., 1985). For example, Clifford (1979; 1980), showed that closing the crop in mid-November rather than mid October or mid-December achieved this objective for NZ bred cultivars. Harvest should then occur at the earliest possible time consistent with quality of the seedlot (Hyde, 1950), usually 6 weeks from peak flowering (Clifford et al., 1985).

(2) Harvest. Seed losses during harvesting of 42-75% (Forster et al., 1962) and 12-39% (Clifford and McCartin, 1985) have been reported. The latter authors surveyed seven crops and recorded a mean loss of 200 kg ha^{-1} which contained 130 kg ha^{-1} of hard seed (range 73-237 kg ha^{-1}). Losses may occur during mowing, at crop pick-up and during threshing as the sequential processes from standing crop to seedlot occur.

Clifford and McCartin (1985) carried out a series of evaluations of mower types and crop conditions for mowing, and found that the hard seed content of seed lost both at mowing and at crop pick-up for threshing ranged from 60-71% - a hard seed return to the soil from the crop pick-up process of 40-210 kg ha^{-1}. Factors affecting hard seed content were not usually consistent between experiments or years (Table 4), although using a rotary mower significantly reduced the hard seed content of pick-up losses in both years (Clifford and McCartin, 1985). There was a large difference in the hard seed content of the offal trail for two combines used (36 v 56% for conventional and axial-flow respectively). Because of this, there were also large differences between the two in distribution of hard seed across the field. For the conventional combine, hard seed deposition was relatively even, while the axial-flow combine deposited nearly three times more hard seed in the offal trail than in the rest of the field (417 v 154 kg ha^{-1} respectively), compounding an already serious problem (Clifford et al., 1985).

Recent work (Clifford et al., 1985) has investigated direct combining of a desiccated crop, using a specially designed rotary mower bank (RMB) mounted on the front of the combine. In a comparison with a rotary-finger type pick-up (Murphy), the RMB prototype has significantly reduced the hard seed content of the seedlots within the harvest process (Table 5). Further work is continuing.

There is an urgent need for improved harvest technology to reduce both seed loss and the hard seed content of the seed returned to the soil. Even using the best techniques available (Clifford et al., 1985), hard seed returned to the soil for a high yielding (1000 kg ha^{-1}) specialist crop is around 70 kg ha^{-1}. Harvest losses provide the major source of contamination within a predominantly cereals/small seeds cropping system.

(3) Chemical manipulation. No satisfactory method has yet been developed to promote either a high level of germination or mortality within the buried seed load. Fumigation with methyl bromide initially broke the dormancy of some hard seed but the method was not considered a suitable treatment for effectively reducing populations of volunteer white clover (Popay et al., 1983). No herbicides are available which can selectively control volunteer legumes in seeded legumes when both are exposed to the herbicide. Rolston et al. (1979) showed that the use of activated carbon applied in bands above the seed row and followed by a non-selective herbicide (e.g. diuron) was a possible method of reducing contamination to acceptable levels, but Clifford et al. (1985) reported a risk of substantial crop damage if heavy rain followed this treatment.

(4) Cultivation and sowing management. Rates of seed softening in buried seed of white clover decrease with increasing depth of burial, apparently because soil insulates seeds from high soil surface temperatures and from fluctuating temperatures (Clifford et al., 1985). Peak softening and establishment from hard seed occurs in the spring (Robinson, 1960), so that seed crops should be early autumn sown to ensure good establishment (Hampton et al., 1987) and reduce contamination from hard seed (Clifford et al., 1985).

Table 4. Harvest factors influencing hard seed content (%) of seed losses returned to the soil (adapted from Clifford and McCartin 1985).

Experiment		Source of seed loss
1	mowing green crop (63%)[a] v mowing desiccated crop (69%)	mowing
2	mowing green crop (76%) v mowing desiccated crop (78%)	mowing
1	rotary mower (60%) v sickle bar mower (71%)	pick-up
2	rotary mower (64%) v sickle bar mower (68%)	pick-up
1	mowing against crop lie (26%) v mowing with crop lie (46%)	offal trail
2	mowing against crop lie (56%) v mowing with crop lie (57%)	offal trail

[a] % hard seed

Table 5. Hard seed content (%) of seed losses within the harvest process

Year	Method	Murphy	RMB	LSD 0.05
1983/84	pick-up	97	76	14.7
	separation	94	44	16.2
1984/85	pick-up	74	47	16.8
	separation	81	61	NS

Rampton and Ching (1970) considered that frequent plowing and deep tillage should be used to bring buried hard seeds to the surface, thus hastening the breaking of dormancy and accelerating the rate of depletion. Suckling and Charlton (1978) showed that four years of cropping (2 years potatoes, 2 years cereals) reduced buried white clover seed levels by over 90%, but there were still around 150 white clover seeds m^{-2} present in the soil. Shillito (1974) advocated the use of minimum tillage, or cultivation only within the top 5 cm to stimulate the germination of hard seeds within this zone. In a study on subterranean clover, Taylor (1985) reported that after four years, residual buried seed levels in the 0-6 cm zone were 20, 240 and 330 m^{-2} for no tillage, minimum tillage and conventional tillage systems respectively. Cultivation continually buried seed, while most of the seed on the surface in the no-tillage plots germinated in the first two years and rapidly depleted seed reserves in the soil. Clifford et al. (1985) compared shallow cultivation (to 10 cm) with direct drilling, but found no treatment differences in the number of contaminant white clover plants per plot.

(5) **Sowing.** Clifford et al. (1985) investigated the effects of sowing rate and row spacing on contamination by volunteer white clover. They precision drilled white clover cv Feathermark (distinguished by a red midrib leaf mark) into a field from which a white clover seed crop (cv Grasslands Huia) had been taken two years previously. The initial buried seed load was 845 m^{-2} which was reduced by 21% following the autumn preparation of a seedbed. By mid-winter, 7% of the original buried seed load was present in the form of established plants (50 m^{-2}), but by early spring, two thirds of these contaminants had perished, leaving 2% of the original buried seed load (19 contaminant plants m^{-2}).

At the conventional 3 kg ha^{-1} sowing rate in 15 cm row widths (Clifford, 1980), 12% of the plants were contaminants. However, the contamination level of the harvested seedlot was 26%. Clifford et al. (1985) explained this difference by noting that leaf size differences as well as competitive advantage were probably contributors to the large increase from percentage vegetative contamination to that found in the harvested seedlot. Lancashire et al. (1985) found that plants derived from the buried seed load appeared to be predominantly smaller leaved than the cultivar from which they were derived, and were thereby predisposed to a greater seed yield potential (Clifford, 1985). Genetic differences in the ability of the two cultivars to produce seed were of no consequence (Clifford et al., 1985).

Contamination within the row was inversely proportional to the within-row seeding rate of the sown cultivar (Clifford et al., 1985). In this trial a sowing rate of 6 kg ha^{-1} reduced the percentage contamination of the harvested seedlot to 13% cf 26% for the 3 kg sowing rate. Increasing the row spacing to 45 cm significantly decreased contamination within the row, but did not reduce contamination of the harvested seedlot, presumably because contaminants within the untreatable 7.5 cm zone either side of the wide spaced rows were better able to exploit the wider inter row area.

Changing Cultivars - Is It Possible?

Clifford et al. (1985) concluded that many uncertainties still remained as to the degree of success most farmers would have when changing cultivars. However in the 1986/87 season, a combination of various sections of the NZ seed industry demonstrated that it is possible to produce seed of alternative cultivars which conforms with OECD certification requirements.

(1) Certification Scheme. After consultation with all sectors of the NZ seed industry, the NZ Ministry of Agriculture and Fisheries (MAF) altered its "standards for cultivars" where the white clover seed crop to be sown was a change of cultivar. The major alterations were: that white clover cannot have been grown in the previous five harvest seasons; the field must have been cultivated annually; a minimum row width of 30 cm; a minimum sowing rate of 3 kg seed ha^{-1}; inspection of the crop by MAF prior to establishment, at the seedling stage, prior to flowering and at flowering (Anon, 1986).

(2) Buried seed testing. The NZ Official Seed Testing Station began a commercial buried seed testing service in 1985. Fields are core sampled to a depth of 5 cm, and buried seed extracted using the method of Hyde and Suckling (1953). Buried seed test results for determining eligibility of fields for certification entry are not compulsory (with the exception of Breeders seed for 'Grasslands' cultivars - Clifford et al., 1985), but most seed companies now have their own buried seed requirements (B. Aitken, pers comm). Lancashire et al. (1985) reported that the average buried seed load of fields submitted for testing to select suitable sites for growing certified breeders seed was 800 m^{-2} (20 seeds per 50 cores), but in 1986, many fields tested had buried seed loads considerably lower than this (J.G. Hampton, unpub data). Suggested standards for breeders seed are 0-120 seeds m^{-2} (406 seeds per 50 cores), and for basic seed 160-240 seeds m^{-2} (0-3 seeds per 50 cores) (Anon, 1987), but the data to support these standards are still being accumulated.

(3) New technology. The NZ Agricultural Engineering Institute in conjunction with a major NZ seed firm has developed an inter-row precision sprayer which allows 80% of the field to be sprayed once with dicamba during the winter. The seed crop is grown in wide rows at high density. Inter-row contamination is removed by herbicide, while a combination of low buried seed levels and high sowing rate reduces the probability of within-row contamination to acceptable levels (de Lacy, 1986).

The Future

Seedlots of European origin grown in NZ under the new system are currently being plot tested as the final check for seed certification. However, the crops from which this seed was produced passed field inspection and preliminary data suggest that the progeny do not differ from plants grown from the breeders' standard seedlot (J.E. Miller, pers comm). The NZ seed industry is confident that it can now produce white clover seed of cultivars wanted outside NZ which meet all OECD requirements.

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