THE DEVELOPMENT OF A WHITE CLOVER FOR USE IN THE EASTERN HIGH POTENTIAL AREAS OF SOUTH AFRICA

by

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DECLARATION

I hereby declare that the results contained in this thesis are from my own original work, unless specifically indicated to the contrary in the text, and have not previously been submitted by me in respect of a degree at any other university.

A. Smith

Pretoria.

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ABSTRACT

THE DEVELOPMENT OF A WHITE CLOVER FOR USE IN THE EASTERN HIGH POTENTIAL AREAS OF SOUTH AFRICA.

The problems associated with the use of white clover in pastures in the eastern high potential areas of South Africa i.e. high P requirements, low tolerance to high Al levels and low pH in the soil as well as a limited survival time of approximately 30 months, were identified and found to be related to the inadequate root system of white clover cultivars.

During the improvement programme cultivars available on the world market were introduced and evaluated under dryland conditions. Selections were made from these introductions on the basis of root conformation in high Al, low pH soils, their response to grazing and induced moisture stress.

A laboratory technique for the improvement of Al tolerance was developed and the tolerance of white clover plants to high levels of Al was improved but due to the complexity of pasture plant improvement it was decided that the selection for tolerance to Al could be more effectively carried out in the field.

The effectiveness of vesicular arbuscular mycorrhizas as phosphate gatherers indicated that local strains of mycorrhizas combined as effectively with white clover as the imported strains.

As no seed production of white clover is undertaken in South Africa guidelines for local seed production were also established.

As a result of the improvement programme, Trifolium repens cv. DUSI was developed as an open pollinated synthetic variety, based on thirty eight selected mother lines. DUSI has a greater tolerance to high Al, low pH, low P in the soil and due to an improved root system with a high percentage of secondary taproots produces better under dryland conditions and has a longer stand life than any of the cultivars of white clover available on the local market.

Plant Breeders Rights were obtained for cv. DUSI and the cultivar was inscribed on the South African variety list. Limited amounts of Breeders seed have been made available to the South African Forage Seed Association for commercial seed production

THE ROOT

Then practically, as you examine plants in detail, ask first respecting them: What kind of root have they?
Is it large or small in proportion to their bulk, and why is it so? What soil does it like, and what properties does it acquire from it? The endevour to answer these questions will soon lead you to a rational enquiry into the plant`s history.
You will first ascertain what rock or earth it delights in, and what climate and circumstances; then you will see how its root is fitted to sustain it mechanically under given pressures and violences, and to find for it the necessary sustenance under given difficulties of famine or drought.

PROSERPINA

VOL.1. 1879.

JOHN RUSKIN

George Allen, Sunnyside, Orpington,Kent. pp. 287.

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CHAPTER 1

GENERAL INTRODUCTION

The Republic of South Africa is in the unique position that both first and third world technology exists side by side, a situation that also applies to agriculture and which has to be taken into account in pasture plant improvement programmes, particularly from the point of view of management of pastures established from such improved strains. Thus although extensive use is being made of improved pastures in many parts of South Africa the actual management of these is often in the hands of people with a very rudimentary knowledge of pasture management and the requirements of the pasture plants. This could be particularly problematical in the case of legume based pastures which normally require a higher standard of management than pastures that consist of a single crop.

The aim of improved pasture utilization is sustained/increased animal production, without loss of the natural resources. The carrying capacity of South African veld has long been overestimated with a resulting depletion and often destruction of the resource. The current state of our natural pastures is such that low quality pastures result in low animal production. It is also found that large areas, previously cultivated to maize, are now reverting back to pasture.

In these areas quality of fodder is a limiting factor to animal production. The quality of natural and cultivated pastures can be vastly improved by the introduction of a legume. Such an introduction can be either as a component of the natural pasture (veld), in which case the legume will have to be established with minimal tillage into a highly competitive plant community, or it could be used, either as a pure stand or a component of a cultivated pasture mixture or pasture system. The cultivated pasture can again be either dryland or irrigated and would normally be used as a strategic feed source of high quality.

Dryland as well as irrigated pasture based on a legume component can be very successfully incorporated in several animal production enterprises such as finishing of weaners, dairying and fat lamb production.

The eastern part of the Republic of South Africa (RSA) receives a relatively high rainfall (Fig.2) which results in it having a high agricultural potential (Fig.3). This high potential is limited to a certain extent by low soil fertility due to excessive leaching of the soils. This poses a particular problem for the inclusion of a legume into pastures.

The economics of pasture establishment and therefore the successful inclusion into farming systems depend largely on factors such as fertility of soils and availability and price of seed of the introduced pasture plants.

It is, however, found that in the case of animal based farming enterprises the capital outlay on implements is often a major cost factor, which can be reduced if:

1. the pastures are perennial and
2. the animal can be used as the only harvester.

Improved pastures are found mainly in the eastern high potential areas of the RSA. However, most of the species used are of Mediterranean origin and as such are not properly adapted to South African conditions. Furthermore the hot moist summers which are conducive to high incidences of disease, the dry, relatively cold winters and poor management coupled to highly leached soils with often very high exchangeable Al, low pH and very low levels of P makes production from pastures a very hazardous enterprise.

When the decision had to be made as to which legume should be used in an improvement programme, several factors had to be taken into account: Although tropical legumes were better suited to the acid soil conditions found in large parts of the high potential areas, other climatic factors such as low radiation and relatively low temperatures in certain areas limited their applicability.

Of the three temperate legumes considered, lucerne, red clover and white clover, lucerne was rejected because of its very low tolerance to acid and high aluminium conditions, and red clover because of the foreseen problems in producing seed in an area where no natural pollinators occur. Furthermore, red clover is weakly perennial and due to the lack of natural seed production would never be able to maintain itself in low cost pastures.

White clover, although it normally has a high moisture and high pH requirement, usually produces large quantities of seed, often with hard seed coats, and shows a large variation in root conformation. Under favourable conditions it is also a long lived perennial with a high tolerance to grazing by both cattle and sheep.

After a clear definition of its shortcomings, i.e. relatively low tolerance to acid and high aluminium conditions, high P requirements and a short, 30 month, survival time in most pastures, a study of the variation that occurs in the genepool for these characteristics clearly showed that an improvement programme to overcome these shortcomings was a real possibility.

If these problems could be overcome it was felt that provided a reliable seed source could be established, a white clover cultivar with persistence under low pH, low P and high Al could become a viable pasture component.

CHAPTER 2

THE EASTERN HIGH POTENTIAL AREAS OF SOUTH AFRICA

The eastern high potential areas of South Africa include the maize producing areas of the eastern Highveld of the Transvaal, parts of the eastern Orange Free State, and large parts of Natal and the eastern Cape. Annual rainfall in many areas exceeds 750 mm per annum (Fig.2) and cultivation of dryland pastures should theoretically be possible. Where the annual rainfall is between 500 and 700 mm it should also be possible to establish improved pastures, provided that supplementary irrigation is available. These could then be used to supplement natural pastures and improve the fodder flow.

An analysis of the situation as it prevails in Natal can be used as an indicator of the importance of the region. Seventy three percent (3,6 m ha) of the white-owned farming land in Natal is used to produce roughage for ruminants (Table 1 and Fig. 1). This area supplies most of the food for 1,3 m beef cattle, 0,97 m sheep and goats and 0,06 m game animals, as well as some of the requirements for 0,22 m dairy cattle and 0,026 m horses.

Currently veld makes up 94%, planted pastures 4% and fodder crops 2% of the 3,6 m ha. However, due to its low production, veld only contributes 60% to the total fodder production, while the contribution from pastures is approximately 25% and that from fodder crops 15%.

Approximately 35% of the large stock and 30% of the dairy cattle are found in the high potential area. In Natal alone one finds 16% of the beef animals and 10% of the dairy cattle of the RSA. Although the number of dairy cattle dropped from 274 000 in 1970 to 220 000 in 1979 there was no reduction in the production of dairy products. This indicates an increase of 14% in productivity during this period, an increase which can be attributed to better management and especially to the strategic supplementation of pasture quality through the use of high quality legume-based cultivated pastures,which increased by 6% during the period mentioned above (Edwards, pers. comm.*).

The 142 000 ha of planted pastures are encountered primarily in bioclimates 3 and 4 (56%) and 6 and 8 (31%)(Fig. 1, Table 1). Sixteen percent of the pastures have a legume component and 18% are irrigated. The most widely used species is Eragrostis curvula in that it makes up 30% of all planted pastures, followed by Pennisetum clandestinum - 24%, Lolium multiflorum - 22%, Dactylis glomerata - 9% and Festuca arundinacea - 5%. Digitaria eriantha is playing an increasing role in the drier areas. Medicago sativa, Trifolium repens and T. pratense are the most important legumes.

* Edwards, P.J. Dept. Agriculture (Natal Region).

Table 1. Area planted to fodder crops and cultivated pastures in various bioclimatic subregions in Natal in 1980, and estimates of areas potentially available (in 1 000 ha).

Parameter	Bioclimatic subregion							
	1	2	3	4 & 5	6	8	7, 9 10 & 11	Total Natal
Area 1980								
Veld	45	146	159	940	627	938	565	3 420
Cult. Past.	2	10	17	63	25	18	7	142
Fodder crops	-	2	6	44	13	16	2	83
Total	47	158	182	1047	665	972	574	3 645
%	1,3	4,3	5,0	28,8	18,2	26,7	15,7	100,0
Estimated potential area								
Veld	5	15	16	103	376	563	554	1 632
Cult. Past.	41	140	156	893	274	389	18	1 911
Fodder crops	1	3	10	51	15	20	2	102
Total	47	158	182	1047	665	972	574	3 645

From Regional development Plan (Natal Region) 1981.

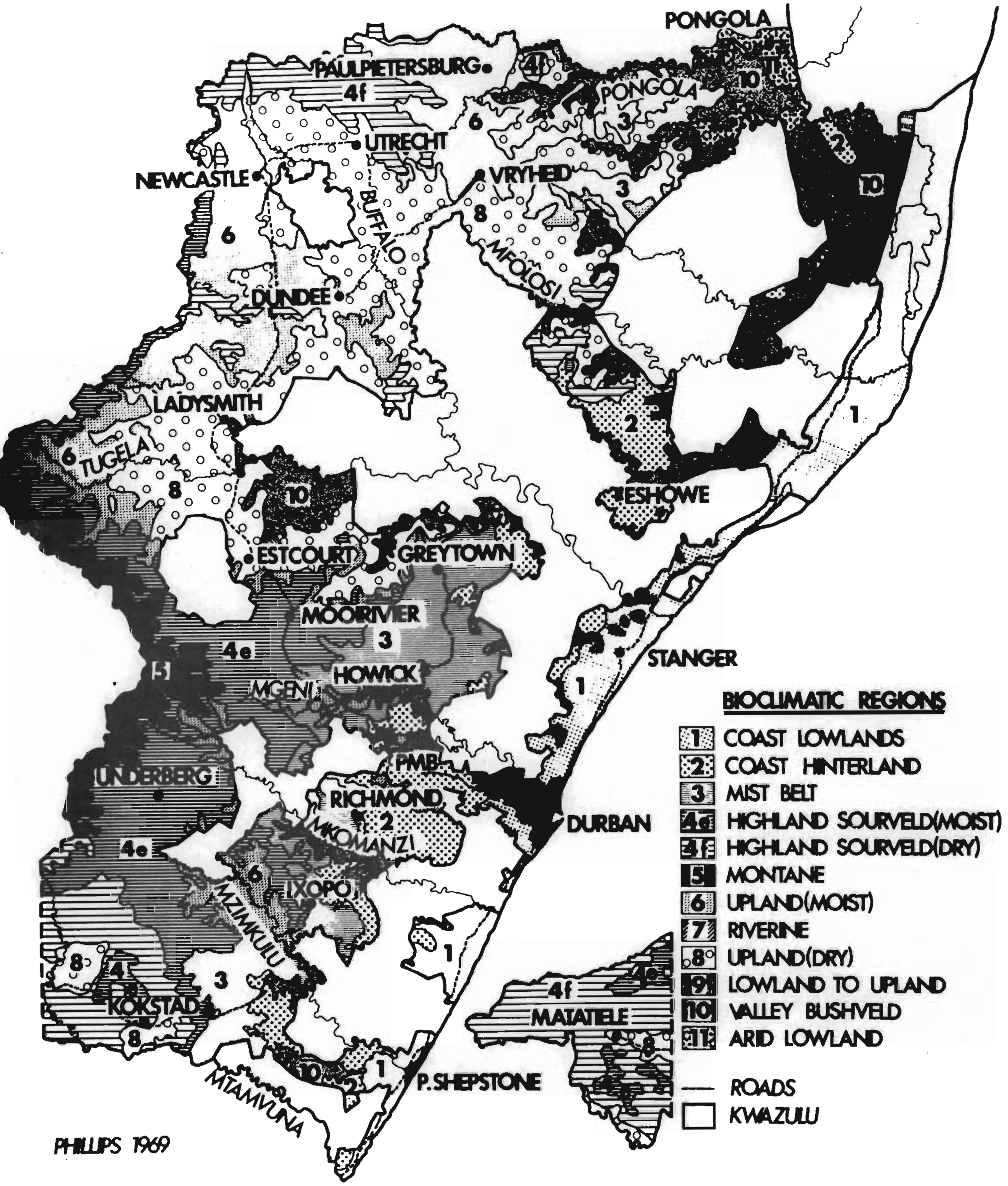

Figure 1. Bioclimatic subregions of Natal.

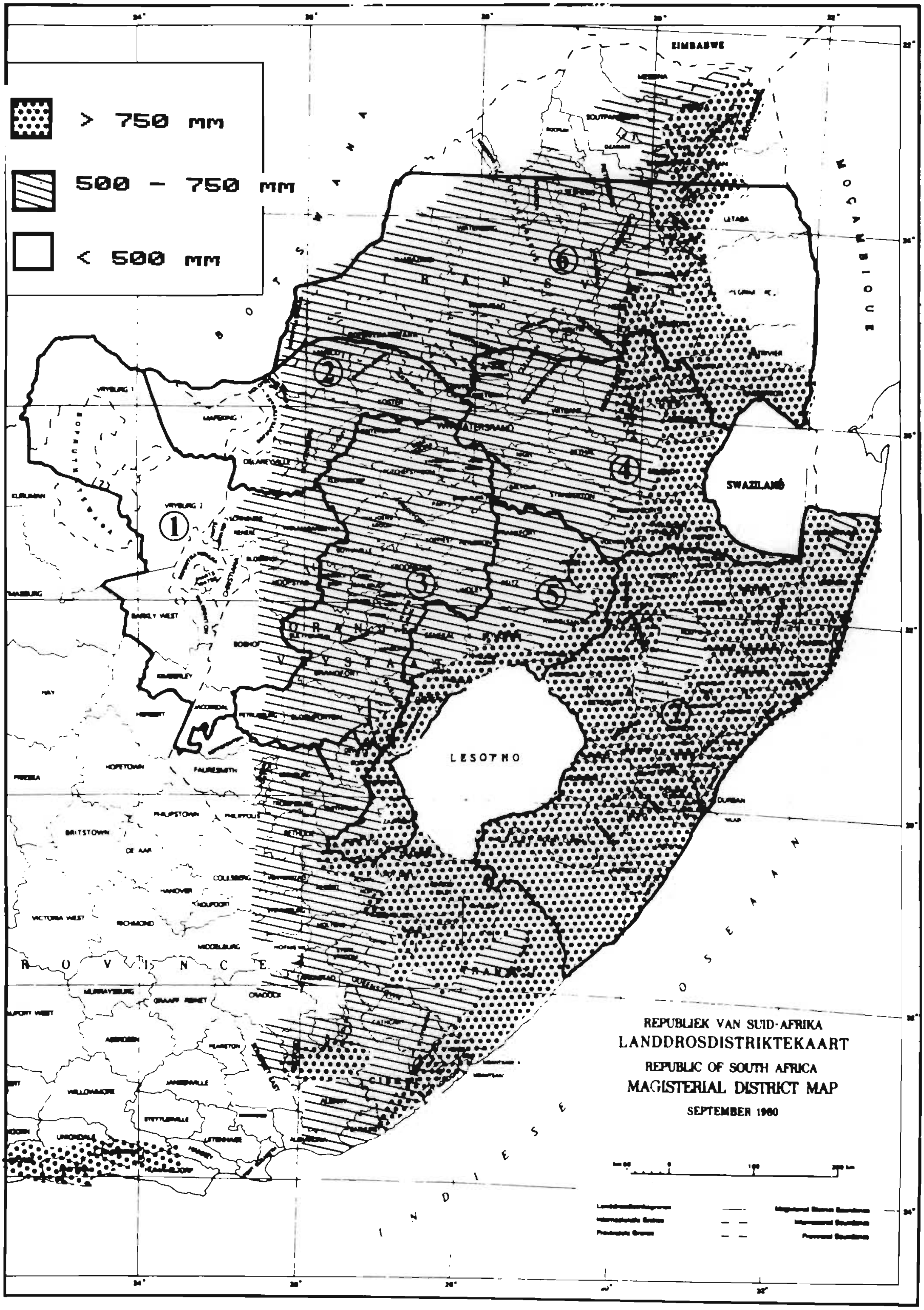

Figure 2. Mean annual rainfall.

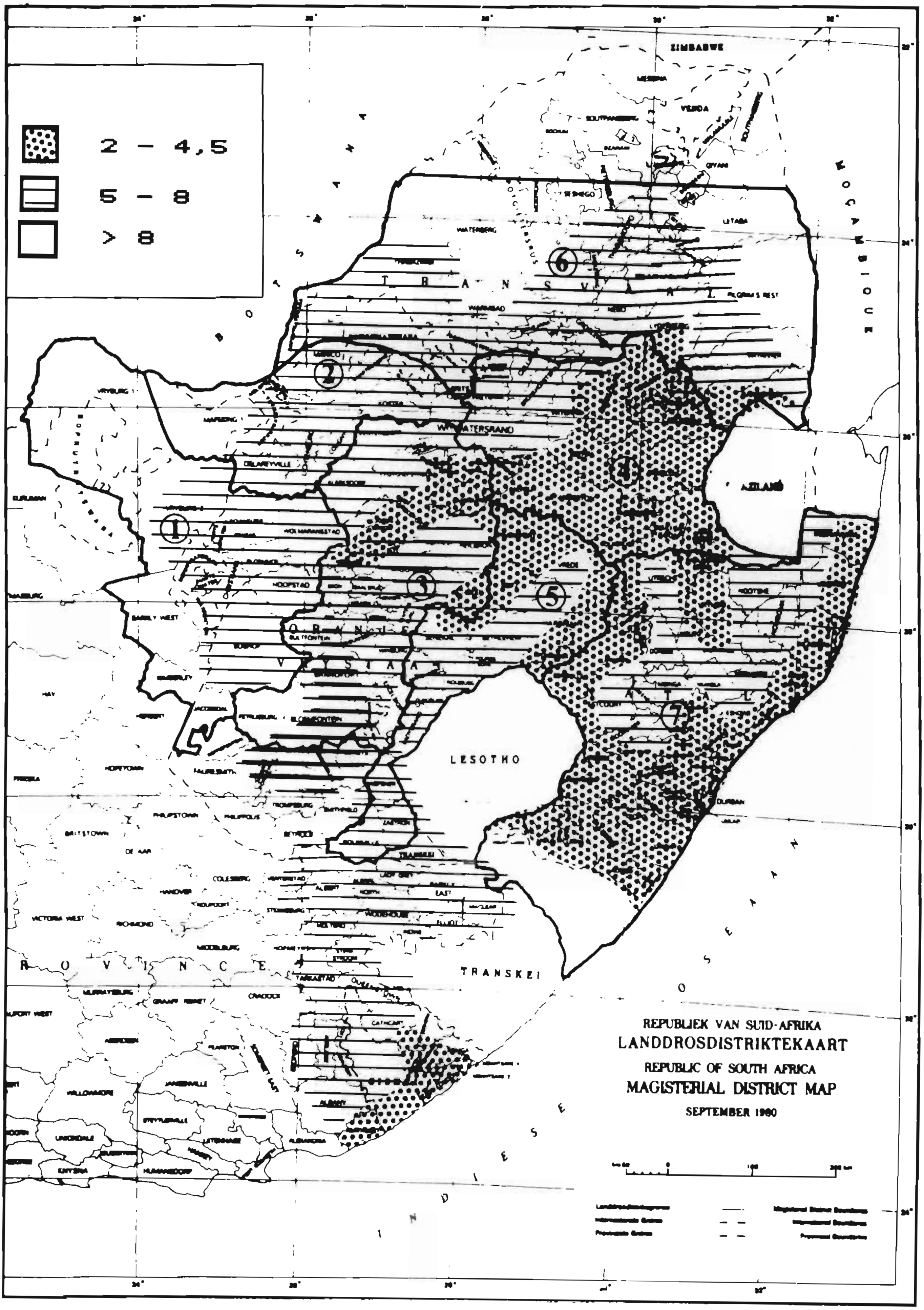

Figure 3 Combined grazing capacities of natural pastures (veld) ha/mlu

From Table 1 it is evident that the potential area for planted pastures in Natal is approximately 1,9 m ha or thirteenfold the area planted in 1980. This area, of which 50% could support legume based pastures, would then supply 92% of the fodder and grazing produced in Natal. Of such potential pasture land probably 95% will occur in the moister bioclimatic regions 3, 4, 6 and 8. Due to the large area potentially available , a tremendous increase in carrying capacity and expansion of livestock numbers to 7 m units or three times the present number is possible.

2.1 SOIL FERTILITY

It is well known that adequate P, K and Ca (together with other meso- and minor elements) are essential for good growth of <u>T. repens</u> (white clover). It is also known that high soil levels of Al, apart from being toxic, also result in the fixation of P; the intensity of which is reduced by lime applications.

In the areas under consideration for the establishment of white clover, soil P levels are generally very low (2-4 ppm in virgin veld) and Al levels often high, particularly in the dystrophic upland situations. K is also sometimes limiting as is S although the latter is usually adequately supplied by the use of single super phosphate.

Of the trace elements Mo, which is important in the symbiotic N fixing process, is easily and cheaply supplied as a seed dressing.

It is therefore apparent that the P, Al, Ca complex is the most critical fertility problem in these areas for T. repens. Adequate P and Ca application can resolve this problem but at a considerable cost. For example we estimate that the typical dystrophic upland soil with 4 ppm P and 2,4 me Al at an acid saturation of 50% would require 1500 kg super phosphate and 9 tons of lime to render it suitable for a permanent stand of T. repens. In order to reduce this cost, band placing of nutrients by sodseeding could be considered, but the differentiation between fertilized and unfertilized areas would then be so great as to severely restrict the spread of the legume. Correct fertilization is essential if production potentials are to be achieved and there will be a strong emphasis on the legume component as a less costly source of N in the mixed pastures. If the pasture plants used do not have improved tolerance to poor soil fertility the low returns from dryland pastures would make high establishment costs, arising from soil amelioration, prohibitive.

2.2 SOIL MOISTURE

Bioclimatic regions 3 and 4 are by South African standards, moist during summer. However, winters are usually dry, while dry spells are also experienced during summer. A white clover with inadequate root penetration requires a constant supply of moisture for production and survival.

2.3 THE SOILS AND LANDSCAPE POSITION

Climate has had an overriding influence over geology in the chemical composition of soils in the bioclimatic regions 3 and 4. Thus the soils are generally dystrophic in the upland situation and on sands, and less highly leached on the bottom lands. The bottom lands due to their surrounds and level nature also tend to be moister than the uplands. Thus except where waterlogging persists one would expect *T. repens* to survive better on bottom lands. This indeed appears to be the case. However, as most of the bottom lands, which are not too wet, have already been developed for irrigated ryegrass or annual crops, expansion for pastures tends towards the uplands, where the more intensely leached and more drought prone sites are less suited to the cultivation of *T. repens*.

CHAPTER 3

THE HISTORY OF PASTURE PLANT IMPROVEMENT IN SOUTH AFRICA

3.1 EARLY ATTEMPTS AT IMPROVEMENT

As far as can be ascertained the first South African to advocate the need for plant improvement in South Africa was F.Blersch, principal of the new Agricultural School at the Victoria College, Stellenbosch. As early as 1888 he spoke about the importance of the science to Agriculture (Blersch,1888) and gave a broad outline of improvement methods. He listed them as follows:

1. the improvement of seed material,
2. the discovery of superior plants,
3. selection and use of accidental variations and
4. the change of seed.

Early evidence of the use of introduced pasture grasses and legumes in South Africa is given by Scott(1896). He reported to the Secretary for Agriculture of the Cape Colony, that after a personal visit to most of the farming districts of the Colony, the Orange Free State, Transvaal, Natal and Basutoland, he was convinced that considerably more use could be made of several temperate grasses and legumes, including white clover. Even at this early stage the need for "good clean seed" was stressed by concerned people like Scott (1896) and Nobbs (1903).

The period 1910 to 1943 saw the establishment of research stations with specific instructions, namely to evaluate and improve pasture species. Research farms at Skinners Court and later Groenkloof in Pretoria were used for early selections on tall fescue and kikuyu (Burt-Davey,1912). In 1923 the Prinshof Grass Breeding Station was established in Pretoria with the main objective of "finding a variety that, in its own particular area, will supply our farmers with a grass which will maintain its feeding value throughout the year"(Anon.,1928). The collection of grasses at Prinshof soon numbered 110 but all efforts to establish a pasture of indigenous clovers proved unsuccessful (Anon.,1928).

Subsequently the work at Prinshof was extended to include the summer grasses Cenchrus, Chloris, Digitaria, Panicum, Pennisetum, Setaria and Themeda, the winter grasses Bromus, Dactylis, Ehrharta, Festuca and Phalaris,(Pole-Evans, 1939), and the legumes Trifolium repens and Trifolium pratense (Pole-Evans, 1937).

Rietondale Research Station which was to take over from Prinshof, was started in 1931 and consisted of an introduction nursery of 1,2 ha while 2,4 ha was devoted to the breeding of pedigree strains of pasture species (Pentz,1932). Although the foundations were laid for an extensive breeding programme on what was considered an outstanding selection of grasses and legumes, staff shortages caused by World War II resulted in the work being suspended in 1943 (Gildenhuys, 1951).

3.2 THE POST WORLD WAR II PERIOD

Grass breeding work was initiated at Potchefstroom in 1946. An early result was the release of the Setaria sphacelata ecotype Kazungula, collected at the junction of the Zambesi and Chobe rivers (Gildenhuys,1951), while Gildenhuys (1950) reported the successful hybridisation of Pennisetum typhoides (pearl millet) and Pennisetum purpureum (elephant grass). The hybrid, later called Bana grass, supposedly yielded up to 90 t/ha of dry matter (DM).

In Natal the selection of strains of Italian ryegrass was started at the Cedara Agricultural College near Pietermaritzburg in 1951 (Cornell, 1951), while considerable expansions of staff and facilities at Rietondale made it the main grass breeding centre in the Transvaal. By 1958, 2 600 varieties of grass were being evaluated and a group of plant breeders was engaged in the development of improved pasture crops (Anon.,1958).

Since breeding of a new pasture cultivar with sexually reproducing species may take up to 15 years, and in view of a severe shortage of seed of suitable cultivars, a decision was taken in the early 1950`s to concentrate on apomictic species such as Eragrostis curvula(cv. Ermelo) and Cenchrus ciliaris (cv. Molopo),this so since both species are prolific seed producers and since selection within such species can be expected to yield results far more rapidly than breeding by conventional methods (Kritzinger, Grobler & Joubert,1961).

Grobler, working with Smith at Rietondale during the 1960`s was involved in breeding programmes on several summer grasses and indigenous legumes,(Smith, 1977) while Smith started some preliminary evaluation and cytological studies on Lotononis spp.(Smith, 1974). Plant improvement work at Rietondale was terminated in 1972 with the transfer of the only remaining pasture breeder (Smith) to Cedara, which then became the only centre for pasture plant improvement in the Republic of South Africa.

3.3 PASTURE PLANT BREEDING AT CEDARA

Although evaluation and selection of Paspalum dilatatum and Lolium multiflorum had been undertaken at Cedara since 1951 in anticipation of the appointment of a pasture plant breeder, the breeding of pasture grasses commenced only in 1959 and that of pasture legumes in 1976.

In April 1959, at a meeting of plant breeders, agronomists and pasture workers at the Natal Agricultural Research Institute, Pietermaritzburg, it was decided that Cedara was to become the principal forage crop improvement centre in the eastern high rainfall region of South Africa. The decision was based on two factors; (1) the favorable location of Cedara - it is situated in the centre of an intensive pasture and ley farming area and it is within close proximity to other research stations in the Natal Region as well as the University of Natal, and (2) because of the relatively favorable summer and winter climatic conditions experienced in the area.

At the meeting a blue print for grass breeding research at Cedara was compiled. A staff of 16 officers (5 professional and 11 technical) was envisaged and it was estimated that a budget of R 120 000 would be necessary to initiate the programme (Kritzinger,1965). Twenty three species of grasses and legumes were selected as desirable material for improvement. Some were widely grown and well known in the Region, others somewhat obscure. Included in the list were *Acroceras*, *Agropyron*, *Agrostis*, *Arrhenatherum*, *Bromus*, *Chloris*, *Dactylis*, *Festuca*, *Hemarthria*, *Holcus*, *Hyparrhenia*, *Lathyrus*, *Lolium*, *Ornithopus*, *Panicum*, *Paspalum*, *Pennisetum*, *Trifolium* and *Vigna*. Needless to say, in view of the shortage of staff and funds a breeding programme as varied as the one proposed, never really stood a chance of success, so that subsequently, emphasis was placed on relatively few species, namely *Chloris gayana*, *Dactylis glomerata*, *Panicum coloratum*, *P.deustum*, *P.maximum*, *Paspalum dilatatum*, *P.urvillei*, *Lolium multiflorum* and *L. perenne*.

As far as legumes in general are concerned no early records of attempts to achieve improvement by breeding are available, while for white clover (Trifolium repens) in particular, no work was undertaken because imported lines appeared to be sufficiently well adapted to the Mist Belt and because overseas institutions reported that little progress was being made in the improvement of the species (Kritzinger,1965).

Nevertheless an improvement programme on T.repens was initiated in 1976 having as main objective, the development of two distinct white clover types to complement the two major companion grasses, kikuyu and tall fescue. Kikuyu requires as companion a medium-leaved, stoloniferous type of clover which produces early in spring, grows fairly vigorously during summer and which can withstand heavy defoliation, wheras tall fescue needs a large-leaved erect type with strong taproot development that can stand a certain amount of shading.However, during 1979 the programme was reviewed and it was decided to concentrate on the development of one type only, namely a white clover with large leaves and a root system adapted to acid soils with a high aluminium content.

3.4 WHY SO LITTLE PROGRESS ?

A question which may well be asked is why so little progress has been achieved with pasture plant improvement in South Africa. The answer lies in the fact that any improvement programme for forage crops is inherently a long term project, taking approximately 10 years for annual and 15 years for perennial species (Anon.,1983).

Furthermore successful breeding is dependent on continuity, both in location and researchers, and is most successful if individual breeders can form part of a strong group of researchers who can complement each other, share facilities, and are located in an area which is favourable for the cultivation of the bred cultivars.

From 1903 to 1956 the South African pasture plant breeders, who were mostly situated in the Transvaal, were located at seven different research stations. By 1956, however, the only breeding programme was at Rietondale, and it is therefore not surprising that progress during the preceding period was minimal.

CHAPTER 4

THE IMPROVEMENT OF PASTURE PLANTS

4.1 THE PURPOSE OF PLANT IMPROVEMENT

In his paper "Plant breeding to what purpose", Keith Campbell (1977) states that:" Plant improvement, whether effected through breeding, selection or introduction, in my view, epitomizes what the application of science to agriculture is all about. I take this to be the production of man`s basic requirement, food, with as great efficiency and regularity as possible".

Campbell claims that the genetic plasticity of species should enable plant breeders to modify and engineer plant structure and function to provide crop types still more efficient and suitable. However, he also warns that one often finds, especially with big groups of plant breeders or in large research institutes, that because of unlimited resources and therefore reduced pressures to supply the goods, that the practical application of breeders lacks quite a bit. <u>One should therefore have clearcut breeding objectives.</u>

Ziman (1976), as quoted by Campbell (1977) states, "it often turns out that scientists who are hired to do applied research are so seduced into open ended basic research that they forget what they are supposed to be doing...... Many of the claims for "socially relevant" research are thoroughly fudged by this method".

The argument is often used that the practical goals can, in some instances, be more efficiently achieved if time is first devoted to a basic understanding of the genetic principles involved. It is, however, true that in some instances this is not necessary, and that these processes can be carried out concurrently to the practical breeding work, especially where due to the time scale involved multiple generations are required to fix new characteristics in plants.

In many parts of the world, the consequences of fiddling, instead of getting on with the job of improving crop productivity are too fearsome to contemplate.

4.2 THE COMPLEXITY OF PASTURE PLANT IMPROVEMENT

The breeding of pasture plants is considerably more involved than that of agronomic crops for the following reasons:

1. When dealing with a pasture plant the whole pasture complex, of animal, plant and soil must be considered.
2. The animal becomes the "mower" or harvester and harvesting is normally done indirectly through the animal. Yields are estimated from animal performance and very seldom can direct estimates be made that would give a true reflection of pasture potential. Animal health and production are of prime importance, and the

pasture plant breeder must take both of these into account when drawing up a model for the intended improved cultivar.

3. The pasture plant must be able to grow in competition with other pasture species. In the case of a legume it must even supply some of the nutrients required by compatible species. It must, in some instances, be perennial, must withstand grazing and often severe mismanagement. The plant must also be adapted to marginal soil conditions, as pastures are more often than not, established on soils not suitable for cash cropping.

4. The soils on which pastures are grown are often of very low nutritive status and the cost of ameliorating these can be greatly reduced by lowering the nutritional requirements of the cultivar being bred.

Other factors that should be taken into consideration are returns to inputs, available manpower, expertise, available resources and urgency of requirement. In all cases the introduction of a well adapted variety or cultivar appears to be the quickest and cheapest way of overcoming existing deficiencies in a pasture crop.

4.3 CHOICE OF A SUITABLE SPECIES

Once the decision has been taken to embark on a breeding programme with a legume, it becomes necessary to identify the species which is best suited to the Eastern High Potential Areas of South Africa and which will be the best candidate for an improvement programme.

Although probably introduced to South Africa in the late 17th century, white clover, as a pasture, is only formally mentioned in agricultural documents by the end of the 19th century. By the early 1900`s we find that white clover is already a well accepted and integral part of improved pastures in Natal, especially in the mistbelt and Drakensberg areas. Furthermore, problems encountered with its cultivation are clearly defined.

Trials in the eastern Cape and eastern Transvaal also indicated its suitability for use as a pasture species as can be seen from the annual progress reports of researchers in these Regions.

Although annual medics grow well in the western Cape they are not adapted to the eastern high potential areas and the use of lucerne is severely restricted by the occurrence of highly leached acid soils with a high exchangeable aluminium content.

From the above T. repens appeared to be the only suitable candidate for a legume improvement programme. Additional reasons why it was decided to work with white clover were the following:

1. Despite the so called "30 month syndrome" resulting primarily from the inherent characteristic of white clover to shed the primary tap root after 12 months, thereby becoming totally reliant on the shallow secondary root system, T.repens is undoubtedly longer lived than T. pratense, which even under

optimum conditions, seldom exceeds three years as a productive crop.

2. It is widely accepted by the farming community

3. Although sensitive to acid conditions, it has a much lower lime requirement than M. sativa.

4. Trifolium repens should be amenable to improvement in terms of its tolerance of low P, Ca, K and high Al soil contents.

5. The main cultivar used in the RSA (cv. LADINO) is not a prolific seed producer and the lack of dormancy and hard seed coats makes seed survival in the soil as a means of re-population after unfavorable conditions and "die out" very limited. The prostrate growth habit is a disadvantage when used in combination with tall companion grasses, although it provides for tolerance to over utilization by most grazing animals.

6. Although game and insects do cause damage to T. repens stands, most of the economical damage seems to be restricted to that caused by diseases, mostly leaf rust and a few virus diseases. The extent of such damage has , however, not yet been quantified.

CHAPTER 5

TRIFOLIUM REPENS (WHITE CLOVER)

5.1 THE TAXONOMY AND CYTOTAXONOMY OF *TRIFOLIUM REPENS*

Trifolium repens Linn. was named by Linnaeus and comprises 6 subspecies, all but one being from the Mediterranean and further east in Europe. Vavilof regards the Mediterranean as the centre of origin. From there white clover spread into Europe to c. 71^{o} N. It was introduced to America in 1750, to Australia and New Zealand c. 1850 and to South Africa in the late 17th Century by the early settlers.

Cytogenetically *T. repens*, 2n = 32, is an amphidiploid with 16 bivalents and mostly disomic inheritance. Fertility is controlled by S factors producing the gametophytic type of incompatibility. Most plants are highly self-sterile with a few pseudo-self fertile and very few truly self-fertile plants. The large number of S alleles present in populations ensure a high level of cross compatibility in natural populations with less than 1% of crosses being incompatible.

5.2 LEAF MARKINGS OF *T.REPENS*

Since leaf markings were used as a marker, or identifying gene in the improvement programme to be described later, they deserve special mention. They consist of white and red patterns, the white V is present in different shapes and shades and is controlled by a series of alleles. Absence of the V marking is recessive to all others. Some of the red patterns form an allelic series independent of the V series, but others are associated with the V locus.

5.3 RHIZOBIA AND MYCORRHIZAS

For T.repens to function effectively as a symbiotic nitrogen fixer it must, at all times, be adequately inoculated with specifically adapted white clover Rhizobia. With the techniques already developed and used in practice, such inoculation does not generally appear to be a problem. Unclear at present is the extent to which the commercial Rhizobium strains are adapted, or will adapt, to low pH conditions.

The effectiveness of P uptake by a plant is directly correlated to the penetration of the soil profile by plant roots. If the root system of a plant can be improved by breeding to penetrate into the acid and aluminium rich layers of soil this P uptake can be greatly improved. Mycorrhiza can act as symbiotic P gatherers and therefore can lower the P requirements of infected plants. The effectiveness of the formation of a symbiosis with local and introduced P gathering mycorrhiza, combined with an improved root system derived from a breeding programme could possibly play an economic role in the effective functioning of a T. repens based pasture.

White clover being a temperate legume has a P requirement which is normally higher than that of its companion grasses.If this requirement could be lowered, by the provision of a more effective root system, to a P requirement closer to that of the grasses a considerable saving can be made on the P fertilizer costs at establishment of the pasture.

5.4 THE GROWTH HABIT AND ROOT DEVELOPMENT OF WHITE CLOVER

Terminology

Caradus (1977) defines taproots as large thick vertically penetrating roots. There are two kinds; the seedling taproot which is formed from growth of the radicle, and the nodal taproot which arises from the nodal or adventitious root. Nodal roots which fail to develop into nodal taproots develop instead into fibrous roots, which are defined as branched roots where no roots are dominant.

The white clover plant develops from axillary buds of a short primary stem of the seedling. New stolons develop from these buds and will normally develop in all directions. The apex of the stolon is indeterminate and usually remains close to the soil surface. Leaves are borne at node positions along these stems, which also root from the nodes.

The primary stem of the original seedling usually dies, together with the seedling taproot, before or during the second year. This heralds the gradual death of the plant unless the stolons which it has mothered are able to survive as independent units, a fact which is also mentioned by Ueno, Yoshihara & Hidaka (1967) and Westbrook & Tessar (1955). The stolons are likely to be able to do so only where conditions are very favorable to white clover growth and where they can produce an adequate root system of their own.

Gill & Vear (1958) describe the root system of white clover as relatively shallow but with considerable variation in the amount of adventitious root production. Caradus (1977,1981) points out that white clover root systems are closely correlated to leaf size; the large-leaved cultivars like G PITAU and LADINO form large taproots with a small percentage of fibrous roots. Small-leaved cultivars, on the other hand, form root systems that have predominantly fibrous roots.

Root system morphology is important in relation to nutrient and water absorption (Ueno & Dorington Williams, 1967; Ueno & Yoshihara, 1967); the more extensive the root systems the greater the ability to take up water and nutrients from the soil. Working on a soil of the Hutton form, Mallet (1972) has shown that a plant with roots reaching down to 60 cm has access to approximately 200% more available soil moisture than a plant with roots penetrating only the top 30 cm of soil.

5.5 MANAGEMENT REQUIREMENTS

During a Symposium held at Cedara on Legumes in Pastures, Tainton expressed the opinion that for adequate management of the clover pasture it is most important that grass growth must be controlled by frequent and moderately heavy grazing, a low level of nitrogen fertilizer and a sufficiency of phosphate, calcium and potash in the soil.

He further states that "We may have to accept, however, that the white clover will have to be re-established in the pasture every second year for it does not have the ability to persist under our conditions. Once the taproot has died and the shallow rooted stolon developed plants are all that remain, irrigation will need to be too frequent to make any reliance on these plants worth while. So one would depend on seedling regeneration, and this is unlikely to be effective in our phosphate fixing soils."(Tainton, 1980)

5.6 IMPROVEMENT OBJECTIVES

Objectives in the improvement of white clover for South African conditions can in large measure be equated to those stated by Mc William (1969), namely:

1. An ability to maintain production over extended periods,

particularly when for reasons of temperature or moisture stress the growth of the associated species is very limited.

2. Ease and reliability of establishment from seed, coupled with a good competitive ability of young seedlings.

3. Persistence under grazing and rapid regeneration after defoliation.

4. Wide adaptability over a range of sites, seasonal conditions and management practices, with a tolerance to stresses imposed by such factors as drought, low temperatures (including frost), waterlogging and soil pH.

5. High seed yields and ease of harvesting.

6. Resistance to diseases and insect pests.

7. Satisfactory quality and freedom from toxic properties injurious to the health of the grazing animal.

8. Efficient nitrogen fixation in legumes coupled with a substantial feedback of nitrogen to the soil system.

9. Compatibility between legume and grass in order to maintain an adequate and vigorous legume component in the pasture, as most studies show that animals do better on legume rich pastures than on a grass pasture of comparable abundance.

10. Ability to respond to applied fertilizers.

Improvement techniques must take into consideration the biology of the plant, the mode of its usage and such factors as the grazing animal and the reaction of the plant to selective grazing. With white clover this also includes its need to be cross pollinated by insects and the wide range of management systems imposed on it as a pure pasture species or as a component of a pasture mixture.

The opinions of renowned breeders seem to be that after initial selection parents must be combined to form potential synthetics. Many suggestions as to how improvement should proceed i.e. from single plants, in mixed swards or under a clipping regime to simulate grazing, (Barklay,1964, Gibson & Hollowell,1966, and Gibson et al 1971) exist. The procedure followed at Cedara, however, was based on the most effective system that could be executed within the severe manpower and monetary restrictions that existed.

During discussions after the Symposium on Legumes in Pastures held at Cedara it was realized that the management principles as well as the shortcomings of white clover as identified by Tainton could be used as guidelines for the construction of the " model white clover for South African conditions". The following were identified as major problems:

1. Lack of persistence due to the dying off of the primary taproot
2. Weak competitive ability with companion grasses
3. High nutritional requirements
4. Intolerance to low pH and high levels of exchangeable aluminium
5. Poor tolerance to drought conditions

All these problems were related in some way to the plants` inadequate root system. The ideal white clover would thus be one that would overcome these defects.

The dying off of the primary taproot should be overcome by the supply of secondary taproots to the resulting secondary plantlets, low competitive ability should be overcome if the roots could penetrate to the deeper layers of soil where water was not limiting, high nutritional requirements should be met by getting a more efficient penetration of the soil profile as well as by an efficient symbiosis with vesicular arbuscular micorrhyza that occur naturally in most soils, and lastly aluminium tolerance could be selected for in the field or the laboratory and Rhizobium symbiosis could be ensured by pelleting seed and by supplying MoO_3 in the pelleting material.

In addition, above ground the plant should have large leaves to be able to compete for light with tall growing companion grasses. Attention should also be given to seed production qualities as large leaved varieties are normally poor seed producers. Due to the adverse climatic conditions which are prevalent in the area where clovers are likely to be established a certain percentage of hard seededness should also be incorporated into the ideal plant.

SECTION II.

ORGANIZATION OF THE IMPROVEMENT PROGRAMME

The improvement programme was organized in the following manner:

1. Introduction and cultivar evaluation

2. Selection procedures and source of breeding materials

3. Testing of locally improved T. repens lines

4. Root development

5. Aluminium tolerance in white clover

6. Vesicular arbuscular mycorrhizas and white clover

7. Development of seed production techniques

8. Registration of the cultivar

CHAPTER 1

INTRODUCTION AND CULTIVAR EVALUATION

During the early part of 1976 a start was made with the introduction of white clover cultivars from as many sources as possible. The OECD (Organization for Economic Co-operation and Development) list of cultivars was used as a reference and cultivars were introduced irrespective of their description or origin, the main objective being to enlarge the then limited genetic base of white clover available in the Republic of South Africa.

The cultivars were introduced in stages and as they arrived at Cedara were included in consecutive yield trials to obtain an indication of their potential and of adaptability. After completion of each trial, ratings were done on survival and selections were made for further use in the improvement programme.

1.1 YIELD TRIAL 1

1.1.1 Materials and Methods

A three replicate randomised blocks experiment with 28 white clover cultivars was established at Cedara on the 8th of March 1977 on a soil of the Hutton form Doveton series (site 1,Table2).

Table 2. Description of experimental sites at Cedara (29^o32'S, 30^o17'E, alt 1067m)

Site	Soil type	Depth of sampling	Soil test P (ug/ml)	Al +H (me/100ml)	Acid sat %	pH (KCl)
1 (C2)	Hutton	10cm	15,0	1,33	21,60	4,20
2 (D3)	Hutton	10cm	12,0	0,18	2,20	4,50
3 (M1)	Hutton	0 -15cm	5,0	1,55	40,26	4,08
		15 -30cm	4,1	1,75	41,07	4,13
		30 -45cm	1,6	1,05	36,33	4,25
		45 -60cm	1,2	0,75	35,71	4,36

Fertilizer was applied according to recommendations from soil tests, and amounted to the following: A basal application of 1 000 kg/ha single superphosphate (8,3% P) and 132 kg/ha muriate of potash (50% K) two weeks before establishment and a topdressing of 120 kg/ha single superphosphate and 150 kg/ha muriate of potash at establishment in order to raise the P test level to 20 ppm and K to 150 ppm. These levels correspond to those normally recommended for the cultivation of pasture grasses and in the case of P are 10 ppm lower than those recommended for pasture legumes.

Prior to planting all seed was washed, dried, inoculated and pelleted with lime. Molybdenum trioxide (MoO_3) at a rate of 300 g/ha was added to the pelleting mixture.

Approximately 25mm water was applied by overhead sprinklers every 14 days throughout the dry autumn and winter months. However, at the onset of the first spring rains during mid September 1977, irrigation was discontinued and this trial was then grown under dryland conditions until it was terminated.

The nett plot size was 1,4 * 7,2 m and all plots were harvested simultaneously with a cutterbar mower set at a height of 20mm when the majority of the cultivars had reached a height of approximately 150mm (Fig. 4). Eight herbage cuts were obtained during the growing season. For the presentation of the data the cultivars were broadly categorized into: large-leaved cultivars (9), medium-leaved cultivars (15) and small-leaved cultivars (4).

1.1.2 Results

Dry matter yields for the 1977/78 season are presented in Tables 3, 4, and 5. The following are noteworthy:

1. As expected, the large-leaved cultivars, with the exception of TAMAR and LOUISIANA S1, outyielded the medium-leaved cultivars which in turn produced more dry matter (DM) herbage than the small-leaved cultivars.(Tables 3, 4 and 5).

2. LADINO, which at this stage is still the most widely used white clover cultivar in South Africa, yielded as much as any other large-leaved introduction under test (Table 3). TONGALA IRRIGATION (Table 4), also available on the local market, although mediocre on a total DM basis, had an interesting growth pattern in that it was one of the highest yielding cultivars in the early parts of the growing season.

3. The peak production period of most cultivars in the large and medium-leaved groups was from September through to January.

4. The cultivars that flowered most profusely, namely TAMAR, LOUISIANA S1 (Table 3), LOUISIANA NSW, CLARENCE and L110 (Table 4), were relatively poor herbage producers. No high yielding cultivars flowered prolifically.

5. Weed infestation ratings carried out in January when weeds first became a problem showed that the degree of weed infestation was always inversely related to the vigour and yielding ability of the cultivars.

Table 3. Dry matter yields (t/ha) of large-leaved *Trifolium repens* cultivars at Cedara during 1977/78.

CULTIVAR	CUT 1	CUT 2	CUT 3	CUT 4	CUT 5
	2/9	29/9	18/10	4/11	28/11
MILLAY	0,82	1,89	1,13	0,99	1,43
CALIF LADINO	0,65	1,67	1,20	1,10	1,52
TILLMAN	0,37	1,15	0,84	0,90	1,53
LADINO	0,37	1,04	0,82	0,89	1,46
ESPANSO (B)	0,11	0,71	0,65	0,97	1,51
SZARVASI 4	0,03	0,39	0,57	0,93	1,49
ESPANSO (A)	0,05	0,52	0,68	0,88	1,40
TAMAR	0,73	1,15	0,56	0,69	0,81
LOUISIANA S1	0,18	0,37	0,38	0,67	1,11

Table 3. (cont.)

CULTIVAR	CUT 6 11/1	CUT 7 3/2	CUT 8 23/3	TOTAL DM YIELD
MILLAY	1,40	1,29	1,16	10,11
CALIF LADINO	1,53	1,25	1,01	9,93
TILLMAN	1,46	1,42	1,74	9,41
LADINO	1,64	1,46	1,72	9,40
ESPANSO (B)	1,62	1,43	1,82	8,82
SZARVASI 4	1,88	1,41	1,82	8,52
ESPANSO (A)	1,58	1,41	1,62	8,14
TAMAR	0,66	0,73	0,00	5,33
LOUISIANA S1	1,20	0,70	0,00	4,61
LSD (0,05)				2,60
LSD (0,01)				3,58
CV (%)				18,00

Table 4. Dry matter yields (t/ha) of medium-leaved *Trifolium repens* cultivars at Cedara during 1977/78

CULTIVAR	CUT 1 2/9	CUT 2 29/9	CUT 3 18/10	CUT 4 4/11	CUT 5 28/11
CRAU	0,04	0,85	0,90	1,05	1,47
KERSEY	0,16	0,98	0,88	0,89	1,29
OLWEN	0,07	0,82	0,96	1,00	1,49
NFG GIGANT	0.01	0,49	0,59	0,76	1,36
VACCARES	0,05	0,64	0,66	0,87	1,24
TONGALA IRR.	0,91	1,64	0,91	0,79	0,85
SVOLDER	0,23	1,45	1,08	0,96	0,99
LIGANTA	0,01	0,34	0,41	0,59	0,98
BLANCA	0,00	0,48	0,53	0,79	0,99
MOTHER WHITE	0,02	0,58	0,64	0,71	1,08
LOUISIANA NSW	0,31	0,79	0,64	0,71	1,03
G HUIA	0,01	0,29	0,52	0,55	1,01
CLARENCE	0,34	1,25	0,46	0,47	0,93
VON KAMEKAS	0,00	0,27	0,40	0,63	1,18
L 110	0,01	0,44	0,38	0,57	1,30

Table 4. (cont.)

CULTIVAR	CUT 6 11/1	CUT 7 3/2	CUT 8 23/3	TOTAL DM YIELD
CRAU	1,34	1,47	1,74	8,86
KERSEY	1,23	1,37	1,69	8,49
OLWEN	1,13	1,26	1,46	8,19
NFG GIGANT	1,36	1,32	1,78	7,67
VACCARES	1,16	1,25	1,36	7,23
TONGALA IRR.	0,97	0,85	0,00	6,92
SVOLDER	0,95	0,99	0,00	6,65
LIGANTA	1,25	1,16	1,70	6,44
BLANCA	1,53	1,27	0,00	5,59
MOTHER WHITE	1,21	1,00	0,00	5,24
LOUISIANA NSW	0,71	0,62	0,00	4,81
G HUIA	1,33	1,02	0,00	4,73
CLARENCE	0,60	0,63	0,00	4,68
VON KAMEKAS	1,34	0,81	0,00	4,63
L 110	1,51	0,19	0,00	4,40
LSD (0,05)				1,87
LSD (0,01)				2,52
CV (%)				18,00

Table 5. Dry matter yields (t/ha) of small-leaved *Trifolium repens* cultivars at Cedara during 1977/78.

CULTIVAR	CUT 1 2/9	CUT 2 29/9	CUT 3 18/10	CUT 4 4/11	CUT 5 28/11
LENA	0,03	0,96	0,79	0,63	0,83
UNDROM	0,00	0,17	0,35	0,57	0,67
S 184	0,00	0,41	0,43	0,33	0,67
PRONITRO	0,00	0,00	0,00	0,01	0,02

CULTIVAR	CUT 6 11/1	CUT 7 3/2	CUT 8 23/3	TOTAL DM YIELD
LENA	1,22	0,79	0,00	5,25
UNDROM	1,18	0,84	0,00	3,78
S 184	1,01	0,69	0,00	3,54
PRONITRO	0,32	0,00	0,00	0,35
LSD (0,05)				2,15
LSD (0,01)				3,57
CV (%)				23,00

Results for the 1978/79 season are presented in Tables 6, 7 and 8. Only four cuts were taken and with the exception of cultivars BLANCA and L 110 the yields in the 1978/79 season were slightly lower than those of the 1977/78 season. Several cultivars did not persist and very low to zero yields were recorded late in the season.

Plants that persisted in the plots of the higher yielding cultivars were selected, seed was collected and used to establish seed production blocks which eventually formed the basis for the improvement programme.

Table 6. Dry matter yields (t/ha) of large-leaved *Trifolium repens* cultivars at Cedara during 1978/79. (cuts 1 to 8 represent the 1977/78 yields).

CULTIVAR	CUTS 1-8 (1977/78)	CUT 9 9/11	CUT 10 13/12	CUT 11 19/01	CUT 12 29/03	CUTS 9-12
MILLAY	10,11	2,00	1,53	1,60	2,04	7,17
LADINO	9,40	1,88	1,40	1,64	1,85	6,77
TILLMAN	9,41	1,74	1,51	1,59	1,69	6,53
SZARVASI 4	8,52	2,09	1,56	1,71	1,95	7,31
ESPANSO B	8,82	1,66	1,57	1,56	2,07	6,86
ESPANSO A	8,14	1,96	1,62	1,51	1,63	6,72
CALIF LADINO	9,89	1,58	1,62	1,55	0,00	4,72
TAMAR	5,33	0,49	0,63	0,00	0,00	1,12
LOUISIANA S1	4,61	0,36	0,99	0,00	0,00	1,35
LSD (0,05)	2,60					0,94
(0,01)	3,58					1,29
CV (%)	18,00					16,30

Table 7. Dry matter yields (t/ha) of medium-leaved *Trifolium repens* cultivars at Cedara during 1978/79. (cuts 1 to 8 represent the 1977/78 yields).

CULTIVAR	CUTS 1-8 (1977/78)	CUT 9 9/11	CUT 10 13/12	CUT 11 19/01	CUT 12 29/03	CUTS 9-12
CRAU	8,86	2,01	1,46	1,46	1,81	6,74
KERSEY	8,49	1,59	1,45	1,34	1,82	6,20
NFG GIGANT	7,67	1,53	1,56	1,48	2,22	6,79
OLWEN	8,19	1,69	1,57	1,14	1,76	6,16
LIGANTA	6,44	1,18	1,55	1,35	2,20	6,28
BLANCA	5,59	1,16	1,71	1,25	2,22	6,34
VACCARES	7,23	1,44	1,36	1,27	0,00	4,07
TONGALA IRR.	6,92	1,24	1,59	1,16	0,00	3,99
SVOLDER	6,65	1,14	1,58	1,33	0,00	4,05
MOTHER WHITE	5,24	1,26	1,60	1,32	0,00	4,18
L 110	4,40	0,63	1,27	1,08	1,63	4,61
G HUIA	4,73	0,63	1,84	1,09	0,00	3,56
CLARENCE	4,68	1,26	1,14	0,00	0,00	2,40
VON KAMEKAS	4,63	0,27	1,05	0,00	0,00	1,32
LOUISIANA NSW	4,81	0,00	0,00	0,00	0,00	0,00
LSD (0,05)	1,87					1,05
(0,01)	2,52					1,42
CV (%)	18,00					18,60

Table 8. Dry matter yields (t/ha) of small-leaved *Trifolium repens* cultivars at Cedara during 1978/79.(cuts 1 to 8 represent the 1977/78 yields).

CULTIVAR	CUTS 1-8 (1977/78)	CUT 9 9/11	CUT 10 13/12	CUT 11 19/01	CUT 12 29/03	CUTS 9-12
S 184	3,54	0,67	1,39	0,95	0,00	3,01
LENA	5,25	0,00	1,19	0,00	0,00	1,19
UNDROM	3,78	0,00	0,35	0,00	0,00	0,35
PRONITRO	0,35	0,00	0,00	0,00	0,00	0,00
LSD (0,05)	2,15					0,99
(0,01)	3,57					1,64
CV (%)	23,00					32,60

Figure 4. Harvesting of white clover trials at Cedara

1.2 YIELD TRIAL 2

1.2.1 Materials and Methods

Yield trial 2 was established as a three replicate randomised blocks trial at Cedara on 13 and 14 April 1978 and included 24 white clover cultivars, twelve of which were also involved in yield trial 1. As with yield trial 1, this experiment was established on a soil of the Doveton form (Hutton series) and fertilizer was applied to attain the required P and K levels as described previously. Annual fertilizer topdressing, seed preparation, irrigation and cutting height treatments were the same as for yield trial 1. Nett plot size was 1,4m *7,2m.

1.2.2 Results

During the first harvest season 5 cuts were taken, the results being presented in Tables 9, 10 and 11. As expected all the large-leaved cultivars outyielded the medium-leaved ones, which in turn outyielded the single small-leaved cultivar.

Yields were, however, considerably lower than those obtained with the same cultivars in the first season of yield trial 1.

Table 9. Dry matter yields (t/ha) of large-leaved *Trifolium repens* cultivars at Cedara during 1978/79.

CULTIVAR	CUT 1 17/10	CUT 2 13/11	CUT 3 6/12	CUT 4 17/01	CUT 5 8/03	CUTS 1-5
ESPANSO	1,88	1,76	1,71	1,44	1,25	8,04
LADINO GIGANTE	1,72	1,42	1,68	1,17	1,24	7,23
TILLMAN	1,57	1,43	1,58	1,36	0,89	6,83
LADINO	2,17	1,63	1,45	1,02	0,47	6,74
SZARVASI 4	1,32	1,31	1,58	1,17	1,18	6,56
LSD (0,05)						2,13
(0,01)						3,10
CV (%)						15,97

Table 10. Dry matter yields (t/ha) of medium-leaved <u>Trifolium repens</u> cultivars at Cedara during 1978/79.

CULTIVAR	CUT 1 17/10	CUT 2 13/11	CUT 3 6/12	CUT 4 17/01	CUT 5 8/03	CUTS 1-5
OLWEN	1,40	1,56	1,48	1,15	0,63	6,22
NFG GIGANT	1,18	1,16	1,39	1,07	0,77	5,57
ULMARA	0,80	1,15	1,61	1,10	0,48	5,14
G HUIA	1,37	1,06	1,28	0,95	0,27	4,93
G 4700	0,94	0,90	1,46	1,01	0,44	4,75
TREVISE	1,04	0,84	1,35	1,18	0,31	4,72
SONJA	1,07	0,91	1,36	0,81	0,53	4,68
TONGALA IRR	1,57	1,03	1,19	0,69	0,07	4,55
SABEDA	0,91	0,88	1,34	0,92	0,38	4,43
G PITAU	1,04	0,83	1,18	0,78	0,51	4,34
BLANCA	0,72	0,79	1,29	1,16	0,36	4,32
KERSEY	0,90	0,80	1,23	0,99	0,39	4,31
SVOLDER	1,14	0,76	1,22	0,87	0,16	4,15
STEINACHER	0,62	0,51	1,18	1,15	0,29	3,75
RETOR	0,71	0,66	1,26	0,77	0,13	3,53
S 100	0,73	0,45	1,18	0,75	0,42	3,53
LIGANTA	0,48	0,53	1,00	1,05	0,40	3,46
PERTINA	0,63	0,42	1,14	0,75	0,15	3,09
LSD (0,05)						1,59
(0,01)						2,14
CV (%)						21,74

Table 11. Dry matter yields (t/ha) of the small-leaved *Trifolium repens* cultivar at Cedara during 1978/79.

CULTIVAR	CUT 1 17/10	CUT 2 13/11	CUT 3 6/12	CUT 4 17/01	CUT 5 8/03	CUTS 1-5
KENT WILD WHITE	0,50	0,00	0,86	0,25	0,04	1,65

Because of the extremely dry conditions experienced during the 1979/80 season and the fact that the trial was conducted under dryland conditions, very few plants in yield trial 2 survived to the second season and consequently no yields were recorded. Only individual plants of cultivars ESPANSO, LADINO GIGANTE, SONJA, TILLMAN and ULMARA showed significant drought tolerance. Seed was collected from these plants and used in the subsequent improvement programme.

1.3 YIELD TRIAL 3

1.3.1 Materials and Methods

Yield trial 3 was established as a three replicate randomised block trial at Cedara on 11 and 12 April 1979 and included 24 white clover cultivars of which 10 were included in previous trials. This brought the number of cultivars screened to 58.

1.3.2 Results

As only 607 mm of rain was recorded during the summer of 1979/80 as opposed to the normal 866 mm, the plants in this trial remained very small and no yields were recorded. Survival rates were very low and the only cultivars that established and survived reasonably well were ARCADIA, LADINO, MERIT, OLWEN, RADI, SZARVASI 4, TILLMAN and TITAN. Seed was collected from promising plants and used in the improvement programme.

A second very dry season during which only 661mm rain was recorded suppressed the yields of all the white clover cultivars,and yield trials 2 and 3 were terminated during the 1980/81 season without any yields being recorded.

1.4 YIELD TRIAL 4

1.4.1 Materials and Methods

During the 1979/80 season 18 additional cultivars were introduced and this brought the total number of cultivars to be evaluated at Cedara to 72. Two local selections, TR 501 and TR 502 were included in yield trial 4, and were treated as cultivars for evaluation purposes, while LADINO was included more than once as the seed originated from different sources. The season was again very dry and only 661 mm of rain was recorded. Yield trial 4 was established as a 7*7 triple lattice at Cedara during April 1980.

1.4.2 Results

Because of the drought, the yields of all the cultivars were suppressed and only four cuts were taken during the season. Yields ranged from 2,2 t/ha to 6,6 t/ha. The two local selections Tr.501 and Tr. 502 were ranked 2 and 4 respectively (Table 12). Both yielded in excess of 6 t/ha DM, and both outyielded all the cultivars then listed on the South African variety list.

Table 12. Dry matter yields (t/ha) of <u>Trifolium repens</u> cultivars evaluated at Cedara during the 1980/81 season.

CULTIVAR	CUT 1 5/11	CUT 2 9/12	CUT 3 8/01	CUT 4 12/02	CUTS 1-4
SZARVASI 4	1,710	2,447	1,341	1,101	6,607
TR 501	1,482	2,312	1,370	1,230	6,361
ESPANSO	1,457	2,298	1,253	1,052	6,087
TR 502	1,637	2,258	1,174	0,994	6,065
MERIT	1,448	2,579	1,020	0,757	5,764
ARCADIA	1,315	2,535	1,241	0,658	5,749
RADI	1,156	2,198	1,221	0,971	5,528
#LADINO	1,281	2,170	1,123	0,925	5,467
CALIF LADINO	0,923	2,186	1,205	1.036	5,329
LUNE DE MAI	0,928	2,254	1,174	0,914	5,274
SHEELIN	1,515	2,214	0,913	0,603	5,237
ROSS	1,809	2,331	0,610	0,441	5,207
#LADINO	0,585	2,177	1,240	1,135	5,137
TITAN	0,862	2,095	1,226	0,908	5,082
ARAN	1,447	2,113	0,804	0,718	5,074
#LADINO	0,418	1,801	1,411	1,436	5,039
TILLMAN	0,309	1,761	1,443	1,486	5,034
LUCLAIR	0,971	1,915	0,211	0,704	5,019

Table 12. (cont.)

CULTIVAR	CUT 1 5/11	CUT 2 9/12	CUT 3 8/01	CUT 4 12/02	CUTS 1-4
ARGENTINA 2	1,903	2,396	0,259	0,255	4,830
LOVASPATONAI	1,504	2,058	0,627	0,445	4,620
LUSTAR	1,251	1,962	0,562	0,671	4,482
MAJOR	0,719	1,977	0,935	0,669	4,312
ARGENTINA 1	1,040	2,431	0,429	0,324	4,247
MIRO OTOFTE	0,978	2,020	0,640	0,590	4,241
KERSEY	1,061	1,808	0,693	0,573	4,143
DONNA	0,588	1,930	0,939	0,588	4,043
NFG GIGANT	0,865	1,705	0,681	0,627	3,915
NESTA	0,883	1,828	0,460	0,701	3,834
WILKLA	0,604	2,021	0,604	0,604	3,833
KARINA	0,971	1,915	0,211	0,704	3,777
DAENO	0,990	1,839	0,580	0,301	3,751
ANIRAK	0,691	1,746	0,501	0,724	3,705
BLANCA	0,213	2,003	0,846	0,534	3,613
OLWEN	0,632	1,734	0,905	0,280	3,532
#PERM PASTURE	0,093	1,332	0,868	1,200	3,465
#TONGALA IRR	0,373	1,872	0,849	0,365	3,461

Table 12. (cont.)

CULTIVAR	CUT 1 5/11	CUT 2 9/12	CUT 3 8/01	CUT 4 12/02	CUTS 1-4
MILKA	1,028	1,614	0,414	0,347	3,407
SVOLDER	0,482	1,663	0,679	0,566	3,366
MILKANOVA	0,721	1,706	0,472	0,449	3,356
G PITAU	0,488	1,704	0,625	0,471	3,301
GANDALF	0,743	1,804	0,452	0,276	3,263
OVCAK	0,294	1,776	0,689	0,549	3,262
LIREPA	0,261	1,700	0,628	0,615	3,229
MENNA	0,450	1,614	0,639	0,435	3,149
#G HUIA	0,170	1,691	0,710	0,476	3,048
NORA	0,698	1,573	0,263	0,354	2,907
CULTURA	0,372	1,443	0,440	0,642	2,862
RIVENDEL	0,313	1,521	0,310	0,512	2,670
BARBIAN	0,049	1,407	0,563	0,235	2,239
LSD (0,05)					1,051
(0,01)					1,440
CV (%)					14,24

ON SA VARIETY LIST

CHAPTER 2

SELECTION PROCEDURES AND SOURCE OF BREEDING MATERIAL

2.1 SELECTION PROCEDURES AND CRITERIA

In view of a critical shortage of qualified plant breeders as well as a lack of funds, the selection methods that were employed to develop a clover suitable for the eastern high potential areas of South Africa had to be somewhat unconventional. Instead of using the normal, relatively sophisticated, breeding techniques employed in other white clover breeding programmes, an approach was adopted whereby natural selection was allowed to exert its effect on large populations of selected plants i.e. those emanating from the yield trials described in the previous section. These were subjected to severe environmental and soil stress factors, in particular low P, low pH and high Al soils.

The fact that the selection was carried out at the Cedara Research Station was particularly advantageous since all the stesses normally encountered by pasture plants in the high potential areas occurred naturally. Rainfall could be controlled by using a movable rain shelter.

The grazing animal formed an integral part of the improvement programme and only after a period of grazing under stress conditions were the final selections made on the basis of root conformation and leaf colour. Making use of a rain shelter, strongly taprooted plants were subjected to severe drought conditions to produce the plants that were ultimately used in the synthesis of the cultivar.

By directing the improvement programme away from that followed by European and New Zealand breeders i.e. away from the requirements set by a Mediterranean climate with mild summers and wet winters, towards hot summers and dry winter conditions with a concomitant change in root conformation from a relatively shallow to a deep-rooted type, good progress was made in a relatively short period of time to obtain a cultivar in which the productive period could be extended considerably above the thirty-month maximum normally associated with white clover in cultivated pastures.

In addition to the ability to survive under stress, the following factors were taken into consideration in the selection of plants for incorporation into the early part of the improvement programme:

1. Visual assessments of individual plants.
2. Yields from cultivar evaluation trials cut on a frequent cutting schedule.
3. Estimates of vigour based on ratings of weed suppression.
4. Ability to withstand grazing by cattle and sheep.

During the final phases of the improvement programme the following additional criteria were used:

1. Virus, bacterial and fungal disease resistance: selection pressures in large populations under stress conditions largely ensure that diseased plants do not survive, and are therefore seldom selected. In the spaced plant situation, however, selection for disease resistance is easily accomplished.

Observations at Cedara in a spaced plant situation indicated that various degrees of rust resistance or rust tolerance exist within the white clover population. Only plants that were free of rust or had a mechanism for limiting the spread of rust, by forming a necrotic spot around the rust pustule, were selected for inclusion in cv. DUSI.

2. Nematode resistance: nematodes are known to cause rapid decline in stands of white clover (Barnes, pers.com.*) and the final selections for cv.DUSI were planted on a field which had a history of nematodes. Inspection of the plants in these selections showed no nematode infection after a full growing season and although no actual testing for nematode resistance was done during the breeding phase, natural variability and selection pressure due to location should ensure a measure of field resistance.

3. Persistence and winter hardiness: although cold hardiness is not a serious factor under South African conditions, survival is definitely influenced by winter temperatures linked to other factors like the weakening of the plants by diseases of the root rot complex and the resulting shallow root system. These factors coupled with poor grazing management and high

grazing pressures help to cause what became known in Natal as the "30 month syndrome".Only by providing the plants with a strong secondary tap root system, through breeding, can plants survive the dry winter conditions.

2.2 THE GENE POOL FOR SELECTION

The gene pool for selection consisted of the following:

1. One block each of large-leaved and medium-leaved plants established from seed collected during December 1977 from the most promising plants of yield trial 1. These were established during 1978.
2. Towards the end of the 1978 season, seed was harvested by hand from the two blocks, bulked, and at the start of the 1979 season was used to establish a large block, approximately 0,2 ha in size at site 2 (Table 12). The area was subjected to frequent grazing by cattle during the productive spring, summer and autumn months. Flowering took place in the pasture during the spring and summer and seed was collected by hand between grazing periods in December 1979, and given the number 501.
3. Seed collected from surviving plants in yield trials 1, 2 and 3 in December 1979, was bulked as line 502. Both lines 501 and 502 were included in yield trial 4 which was established in the autumn of 1980. The remaining seed of line 502 was planted in an

* Barnes, D.L. Dept. of Agriculture (Transvaal Region).

area adjacent to the grazing area and the seed harvested from this combined area in December 1980 was numbered 503. Seed harvested from this same area in December 1981 was given the number 504 and used in later yield trials.

4. In the autumn of 1982, seed from batch 503 was established on site 3 (Table 2), which is characterized by very high acid saturation percentages. These if present in excess of 18% severely reduce white clover production according to Miles, Bartholomew, Macdonald & Kirton, (1982). Furthermore the high aluminium content of the soils severely inhibits root growth and nodule formation in commercially available cultivars.

Contrary to the commercial lines, examination of the root system of line 503 grown at site 3 indicated that a healthy root formation had taken place throughout the whole soil profile (see Fig.17). Fibrous roots were abundant in the top 10 to 15 cm and the plants had a highly developed nodal taproot system which penetrated the subsoil to a depth of more than 60 cm. Active nodules were found on roots up to 30 cm below the soil surface.

On the basis of vigour and absence of leaf markings single plants were selected from this block as well as from the grazing block, individually dug up and inspected for a desirable root conformation. Only plants with strongly developed secondary taproots were finally selected and transplanted to an area under a movable rain shelter in December 1982. The plants were established with irrigation after which a rain shelter, a plastic tunnel on rails, was used to exclude any precipitation that occurred during the following 18 months. No irrigation was applied to the plants during this stress period. A large number of the deep rooted plants survived the treatment and produced seed which collectively was given the number 506 and which together with line 504 was tested in yield trials 5 and 6. Line 504 produced well but did not have the same root development as line 506, and therefore did not persist as well in the trials. These trials were abandoned in the second season due to the very poor survival of the cultivars S 100 and S 184.

Seed of line 506 was also used to establish a nursery of approximately 800 single plants in April 1984. On the basis of various criteria, including root conformation, leaf marking and disease tolerance only 38 plants of the original 800 were selected and vegetatively transferred to establish a seed production block in December 1984.

Seed from this block was designated DUSI and entered into yield trials 7 and 8 at Cedara, some seed was also submitted to the Directorate of Plant and Seed Control to be entered into the required tests for the registration of the cultivar, as well as for the granting of Plant Breeders Rights, according to the Plant Breeders Rights Act of 1976.

CHAPTER 3

TESTING OF LOCALLY IMPROVED T.REPENS LINES

3.1 YIELD TRIALS 5 AND 6

3.1.1 Materials and Methods

During April 1984 yield trials 5 and 6 were established at Cedara to evaluate the advanced breeder lines Tr 504 and Tr 506. These lines were entered into the trials with the cultivars LADINO, S 100, S 184 and G HUIA (HUIA), these being the only cultivars registered on the South African variety list,and of which there was any seed available on the market at that stage.

The trials were duplicates conducted under the same climatic conditions, but whereas yield trial 5 was irrigated, yield trial 6 was under dryland conditions.

Both trials were established as randomised blocks experiments with four replicates, with a sowing rate of 3 kg/ha. Nett plot sizes were 7,2m * 1,4m and both trials were watered through the establishment stage until the onset of the first spring rains. The recorded rainfall for the 1984/85 season at Cedara was 884,80 mm. and fertilizer treatment was the same as for yield trials 1 to 4. Six cuts were recorded during the growing season at approximately 4 weekly intervals.

3.1.2 Results

Yields of the cultivars are represented in Tables 13 and 14.

Table 13. Dry matter yields (t/ha) of white clover cultivars under irrigation at Cedara during the 1984/85 season.

CULTIVAR	CUT 1 02/10	CUT 2 30/10	CUT 3 27/11	CUT 4 27/12	CUT 5 29/01	CUT 6 14/03	TOTAL 1 TO 6
TR 506	1,687	1,430	1,520	1,693	1,168	0,488	7,986
S 100	0,977	0,329	0,538	0,630	0,379	0,643	3,496
LADINO	2,167	1,440	1,180	1,280	0,740	0,484	7,290
G HUIA	1,736	0,604	0,624	0,646	0,439	0,649	4,697
S 184	0,885	0,355	0,508	0,568	0,417	0,645	3,378
TR 504	1,401	1,341	1,554	1,799	1,205	0,632	7,931
LSD (0,05)							1,721
LSD (0,01)							2,001
CV (%)							19,34

Table 14. Dry matter yields (t/ha) of white clover cultivars under dryland conditions at Cedara during the 1984/85 season.

CULTIVAR	CUT 1 02/10	CUT 2 30/10	CUT 3 27/11	CUT 4 27/12	CUT 5 29/01	CUT 6 14/03	TOTAL 1 TO 6
TR 506	1,879	1,462	1,691	1,351	0,660	0,617	7,661
S 100	1,135	0,614	0,580	0,335	0,295	0,768	3,727
LADINO	2,739	1,510	1,506	1,324	0,779	0,796	8,653
G HUIA	1,714	0,790	0,749	0,241	0,231	0,835	4,560
S 184	0,659	0,394	0,423	0,236	0,219	0,795	2,726
TR 504	1,105	1,164	1,464	1.270	0,774	0,742	6,517
LSD (0,05)							1,657
LSD (0,01)							2,324
CV (%)							24,23

The results indicate that the local selections are producing DM yields equal to those of cv LADINO , and much higher yields than the British and New Zealand cultivars.

3.2 YIELD TRIALS 7 AND 8

3.2.1 Materials and Methods

DUSI was included as a cultivar into yield trials 7 and 8, and line 504 was excluded. The climatic conditions at Cedara were slightly more favorable during the 1985/86 season with a rainfall of 807 mm being recorded. The experimental layout and procedures for yield trials 7 and 8 were similar to those for yield trials 5 and 6, with the difference that seven cuts were taken during the season.

3.2.2 Results

The yields are presented in Tables 15 and 16.

Table 15. Dry matter yields (t/ha) of white clover cultivars under irrigation at Cedara during the 1985/86 season.

CULTIVAR	CUT 1 14/08	CUT 2 11/09	CUT 3 11/10	CUT 4 06/11	CUT 5 04/12	CUT 6 02/01	CUT 7 06/02	CUTS 1 TO 7
DUSI	2,229	1,500	1,102	1,026	1,925	0,847	1,437	10,066
TR 506	1,191	1,193	1,347	1,252	2,074	1,221	1,594	9,870
S 100	0,678	0,891	0,954	0,605	1,253	0,799	0,375	5,556
S 184	0,798	0,816	1,221	0,582	1,350	0,811	0,272	5,850
LADINO	2,333	1,487	0,937	0,945	1,858	1,065	1,215	9,839
G HUIA	1,500	1,117	1,018	0,687	1,406	0,811	0,414	6,953
LSD (0,05)								1,111
LSD (0,01)								1,432
CV (%)								13,68

Table 16.Dry matter yields (t/ha) of white clover cultivars under dryland conditions at Cedara during the 1985/86 season.

CULTIVAR	CUT 1 14/08	CUT 2 11/09	CUT 3 11/10	CUT 4 06/11	CUT 5 04/12	CUT 6 02/01	CUT 7 06/02	CUTS 1 TO 7
DUSI	1,796	1,190	0,728	0,826	2,307	1,309	1,649	9,777
TR 506	0,615	0,493	0,542	0,516	2,006	1,064	1,470	6,705
S 100	0,068	0,198	0,375	0,114	1,306	1,218	0,223	3,501
S 184	0,195	0,499	0,486	0,086	1,376	0,718	0,147	3,506
LADINO	1,763	1,181	0,655	0,660	2,129	1,267	1,280	8,894
G HUIA	1,485	1,091	0,678	0,415	1,719	0,861	0,358	6,607
LSD (0,05)								1,102
LSD (0,01)								1,651
CV (%)								22,17

It is clear that under both dryland as well as irrigated conditions, the yield of cv. DUSI compares favorably with those of registered cultivars. In both trials there was a slight problem with slow germination of cv. DUSI seed due to hard seededness. The yields of this cultivar may thus have been slightly depressed in the first harvest, but by the second harvest there was no apparent lowering of the yields, although the stand was less dense than that of cv. LADINO. During the latter part of the season and the season following establishment the stand had, however, thickened up considerably.

At the end of the first productive season with the weakening of clover stands it is very obvious that relative competitiveness of the cultivar can be directly related to the suppression of weeds in the stands.

In Figures 5 to 10 (irrigated) and 11 to 16 (dryland) the aggressiveness of cv. DUSI and line 506 is clearly indicated. This ability to suppress grass as well as broadleaf weed invasion ensures a much longer productive life for DUSI based pastures.

The stand of white clover in yield trial 7 after the second winter.

Figure 5. cv. DUSI

Figure 6. line 506

The stand of white clover in yield trial 7 after the second winter.

Figure 9. cv. S 100

Figure 10. cv. S 184

The stand of white clover in yield trial 8 after the second winter.

Figure 11. cv. DUSI

Figure 12. line 506

The stand of white clover in yield trial 8 after the second winter.

Figure 13. cv. LADINO

Figure 14. cv. G HUIA

The stand of white clover in yield trial 8 after the second winter.

Figure 15. cv. S 100

Figure 16. cv. S 184

CHAPTER 4

ROOT DEVELOPMENT

4.1 SELECTION FOR SECONDARY TAPROOT DEVELOPMENT

To compare the root development of line 501, a selection used in the improvement programme at Cedara, with that of other cultivars on the South African variety list, measurements were made on plants from a 26 month old cultivar trial grown under dryland conditions at Cedara. Soil on this site is of the Hutton form Doveton series, with a pH (KCl) of 4,20, P (ug/ml) of 15, Al+H (me/100ml) of 1,33 and an acid saturation percentage of 21,6%. The number and mass of nodal taproots and the mass of fibrous roots was determined and compared with that of five cultivars, LADINO, HUIA, PITAU, PERMANENT PASTURE and TONGALA IRRIGATION (IRRIGATION). Plants randomly selected from surviving plots of these cultivars were dug up taking sods to a depth of 30 cm and the root systems were carefully washed and air dried. The oldest 10 cm section of each tiller was taken to determine the number and mass of nodal taproots and the mass of fibrous roots.

The results are presented in Table 17.

Table 17. Comparison of white clover root systems.

CULTIVAR	MEAN NO OF NODAL TAPROOTS/10 CM.	MASS OF NODAL TAPROOTS gm/10cm	MASS OF FIBROUS ROOTS gm/10cm
LADINO	3,6	0,3395	0,2898
G PITAU	3,0	0,4490	0,3168
G HUIA	2,4	0,2495	0,2985
PERM. PASTURE	1,8	0,3132	0,3467
IRRIGATION	0,0	0,0000	0,6454
TR 501	4,7	1,2613	1,1937
LSD (0,05)	0,5524	0,1833	0,2477
(0,01)	0,7357	0,2442	0,3298
CV (%)			6,8

LADINO, HUIA and PITAU have been described by Caradus (1977) as large-leaved, taprooted cultivars, whilst PERMANENT PASTURE and IRRIGATION, being medium-leaved cultivars, would be expected to have root systems dominated by fibrous roots (Caradus 1982). Data clearly indicate that the selection 501 has significantly more nodal taproots than any of the other five cultivars tested. Furthermore, the effectiveness of this highly developed root system is demonstrated by the relatively high yields obtained under dryland conditions, see Table 18.

Table 18. Yield of white clover cultivars under dryland conditions at Cedara during 1980/81. **

CULTIVAR	DM YIELD (t/ha)
LADINO	5,214
G HUIA	3,048
G PITAU	3,301
PERM. PASTURE	3,465
IRRIGATION	3,461
TR 501	6,213
LSD (0,05)	1,052
(0,01)	1,441
CV (%)	13,06

** Annual rainfall July 1980 to June 1981 = 661,9 mm.

In addition, line 501, under irrigation at Cedara yielded from 13 to 15t DM/ha in 1981 (Smith,1982). This would indicate that the root system, although primarily developed for dryland conditions, also functions extremely well under irrigation.

4.2 ROOT CONFORMATION OF CULTIVARS IN HIGH ALUMINIUM SOILS

In order to study and compare the root development of DUSI with that of other cultivars used in South Africa, the various cultivars were established on a highly leached Hutton soil at Cedara, which is known to be highly unfavourable to clover growth. Soil tests of the area are given in Table 19.

Table 19. Soil tests of the area used for clover root studies.

SOIL TEST	TOP SOIL	SOIL DEEPER THAN 15cm	IDEAL
P ppm	2	1	30
K ppm	138	122	140
Ca ppm	411	311	1 000
Mg ppm	112	160	80
Na ppm	37	43	---
Zn ppm	0	0	---
Al ppm	100	174	---
pH (KCl)	4,1	4,0	6,0
Acid sat %	24,1	36,5	<3

Phosphate levels were adjusted to 15 ppm (Hunter) and the plots were split for lime, applied at 7 t/ha. The experiment was successfully established using seedlings of the different cultivars, and by hand watering seedlings until the early spring rains. The cultivars tested were LADINO, HUIA, PITAU, IRRIGATION and DUSI.

By using a device adapted from a design of Schuurman and Goedewaagen (1965), root profiles of the different cultivars were obtained. These indicated root penetration in excess of 600 mm in acid soils for DUSI, and also that nodulation can take place as deep as 350 mm below the soil surface given suitable host roots. Plants within cultivars differed remarkably little in root development but marked differences existed between cultivars (Figs. 17 and 18).

Measurements were taken of the following plant characteristics:

1.Spread from center of plant in cm.

2. Primary taproot,

    size (rated on a 1 to 5 scale), 1 = small, 5 = large

    lesions (1 to 5) 1 = few, 5 = many

    length of longest branch in cm.

    average length of branches in cm.

3. Secondary taproots,

    number (1 to 5) 1 = few, 5 = many

    length of longest in cm.

    average length in cm.

4. Nodulation (1 to 5) 1 = few 5 = many

5. Yield, of a 15cm strip taken across the center of the plant,

leaves, green mass,
dry mass,
stems, green mass,
dry mass,

primary taproot, green mass,
dry mass,
secondary taproots, green mass,
dry mass,
fibrous roots, green mass,
dry mass.

Data obtained from rooting profiles and yields of those plants harvested were meaned and used to obtain a graphic representation (Fig. 19) of the plants growing under limed and acid conditions. These are given in Figs. 20 to 24 for the different cultivars.

Figure 17. Root system of cv. DUSI.

Figure 18. Root system of cv. LADINO

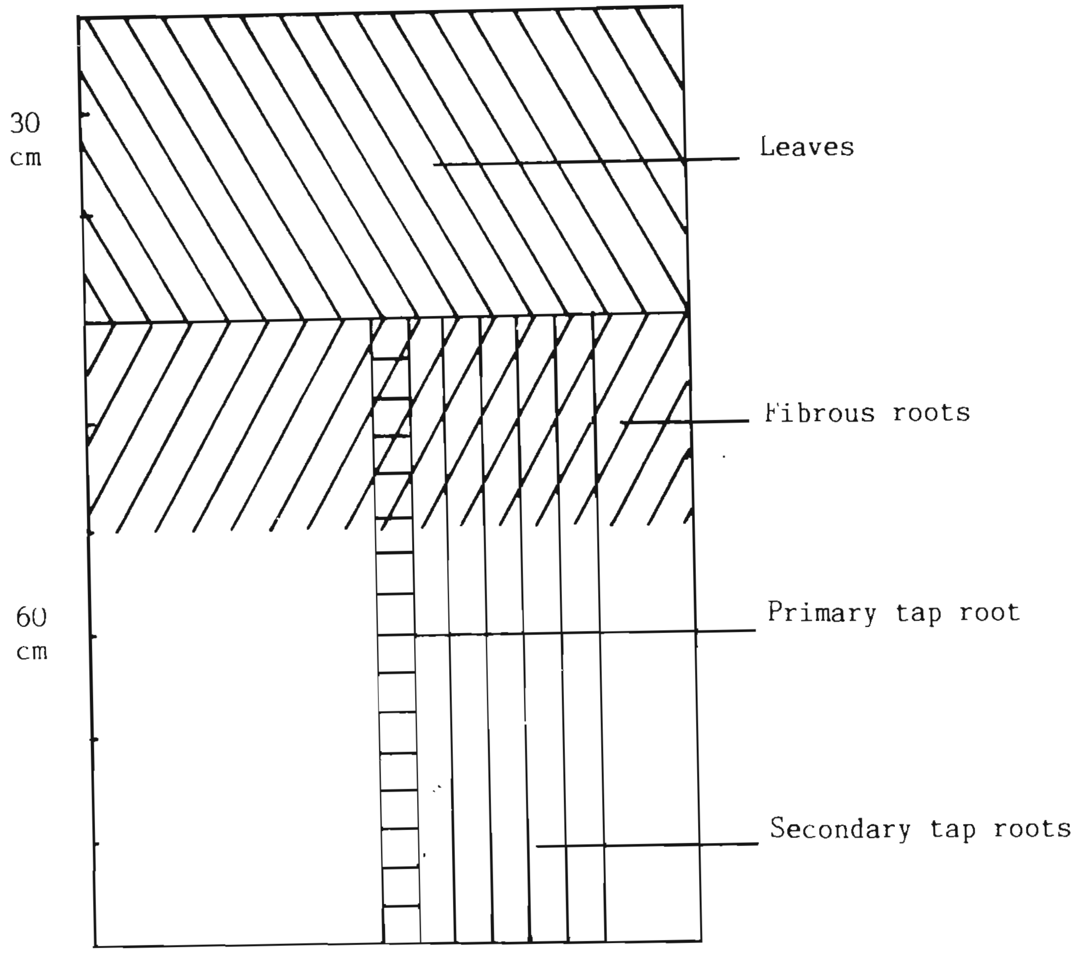

Figure 19. Graphic representation of white clover root development and herbage production.

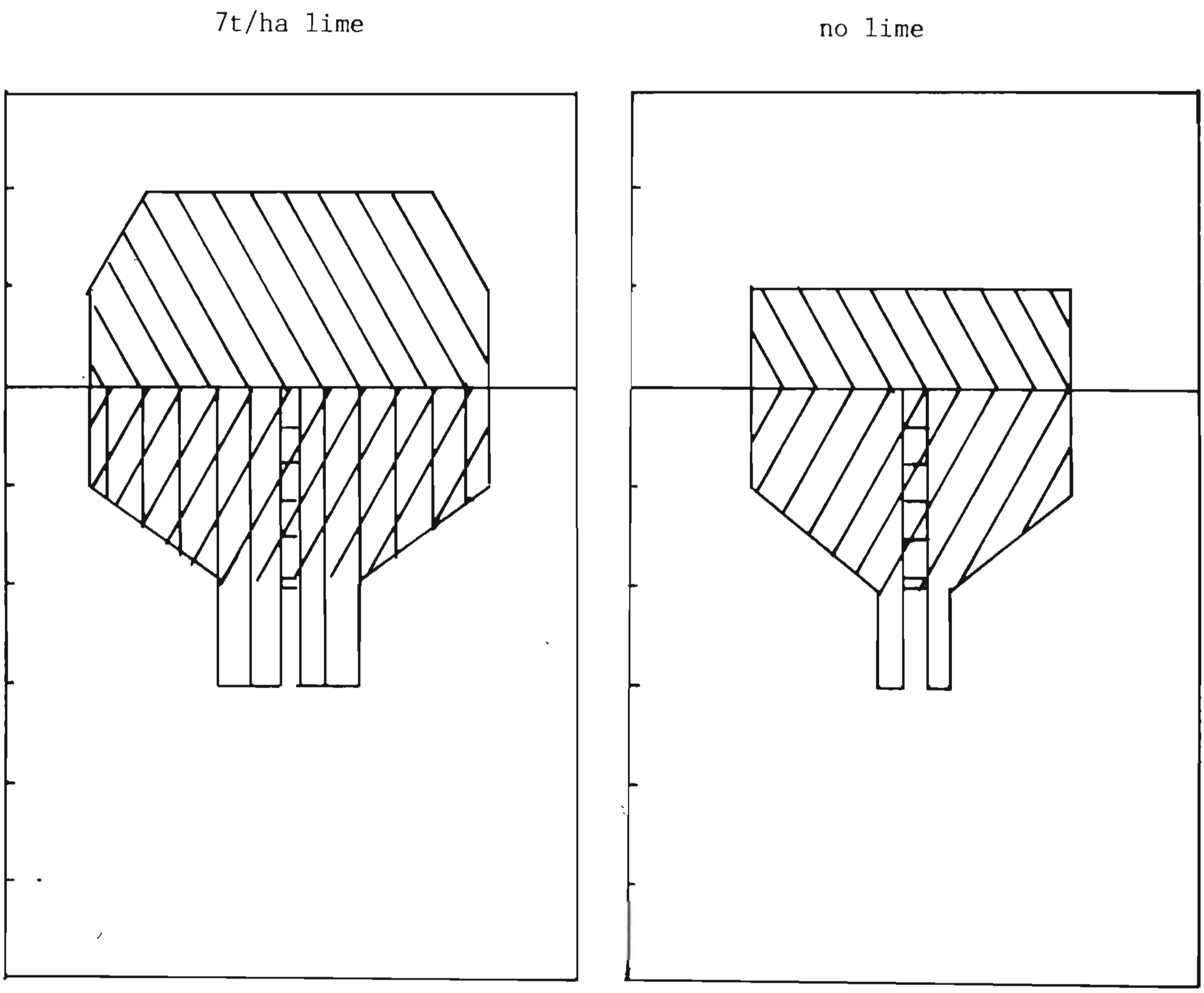

Figure 20. Development of cv. PITAU in acid soils with and without lime application.

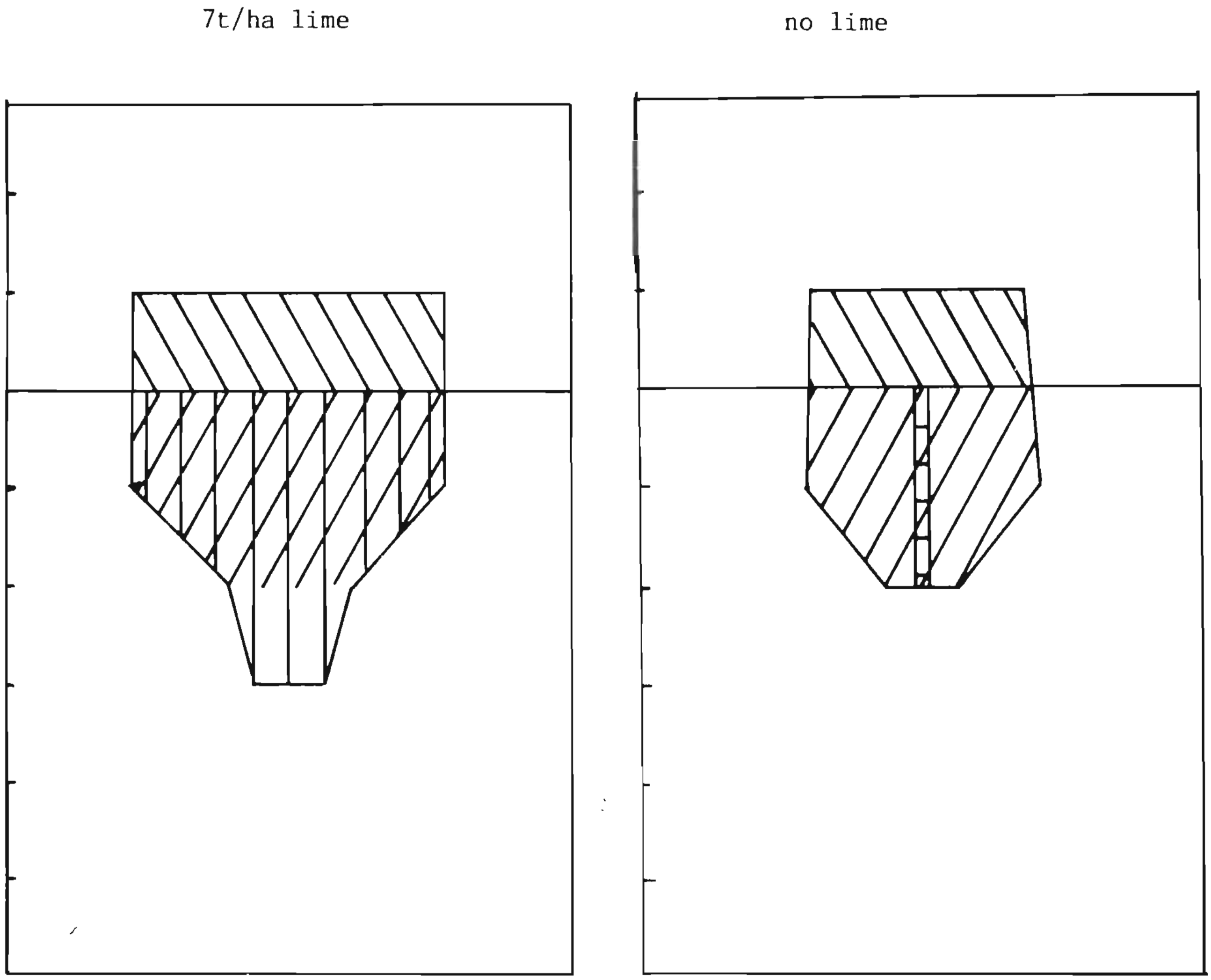

Figure 21. Development of cv. HUIA in acid soils with and without lime application.

/t/ha lime no lime

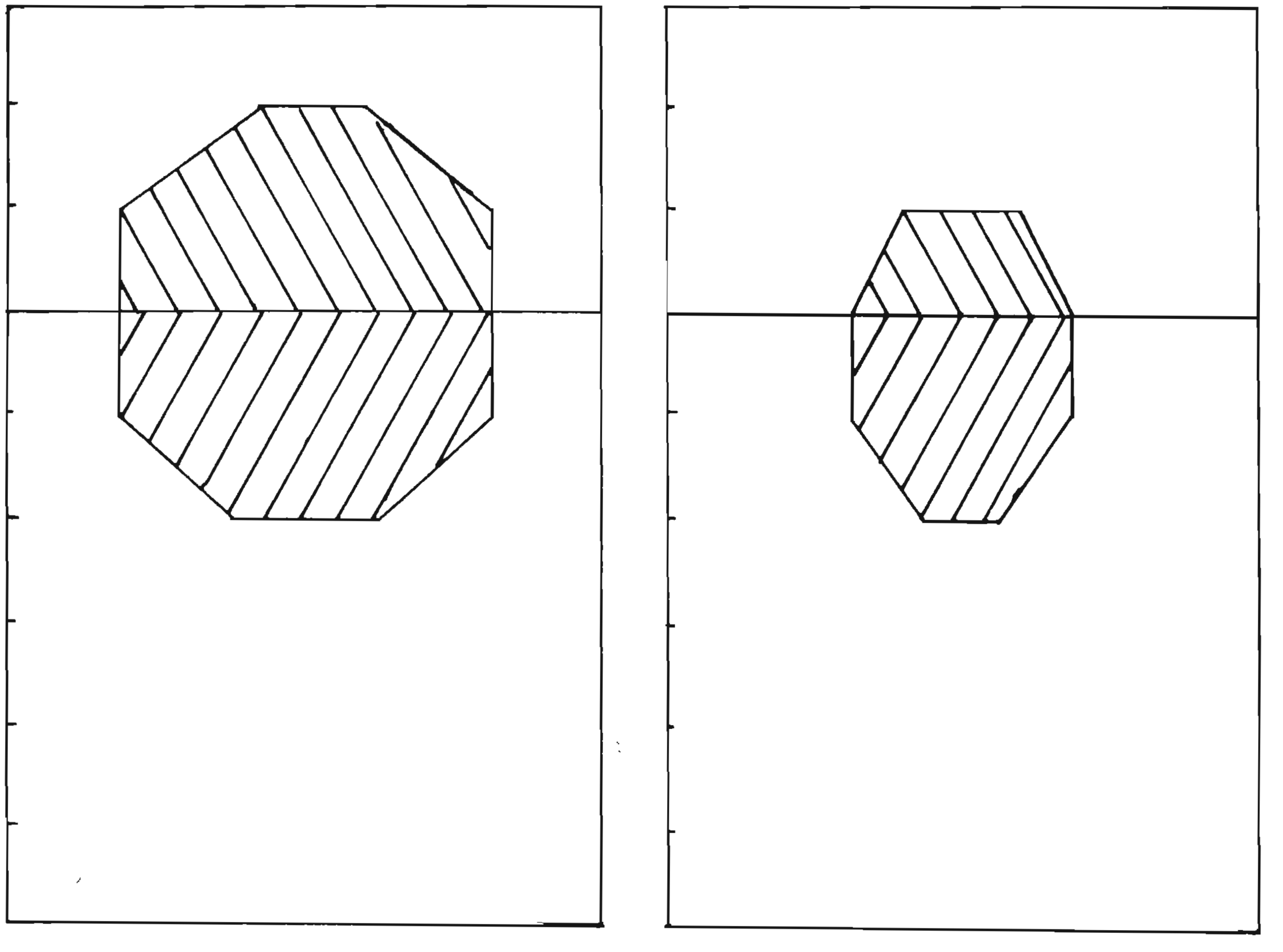

Figure 22. Development of cv. IRRIGATION in acid soils with and witout`lime application.

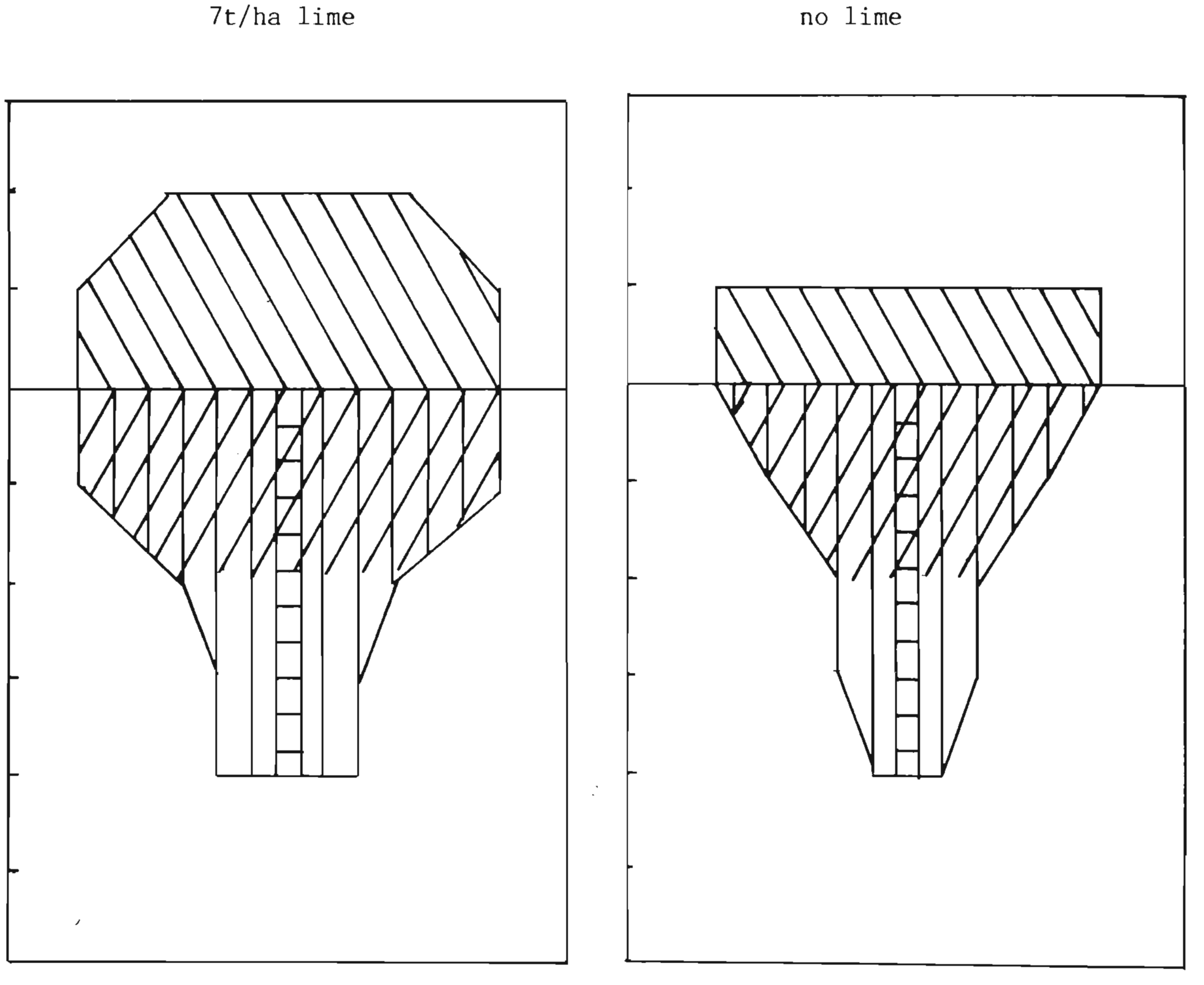

Figure 23. Development of cv. LADINO in acid soils with and without lime application.

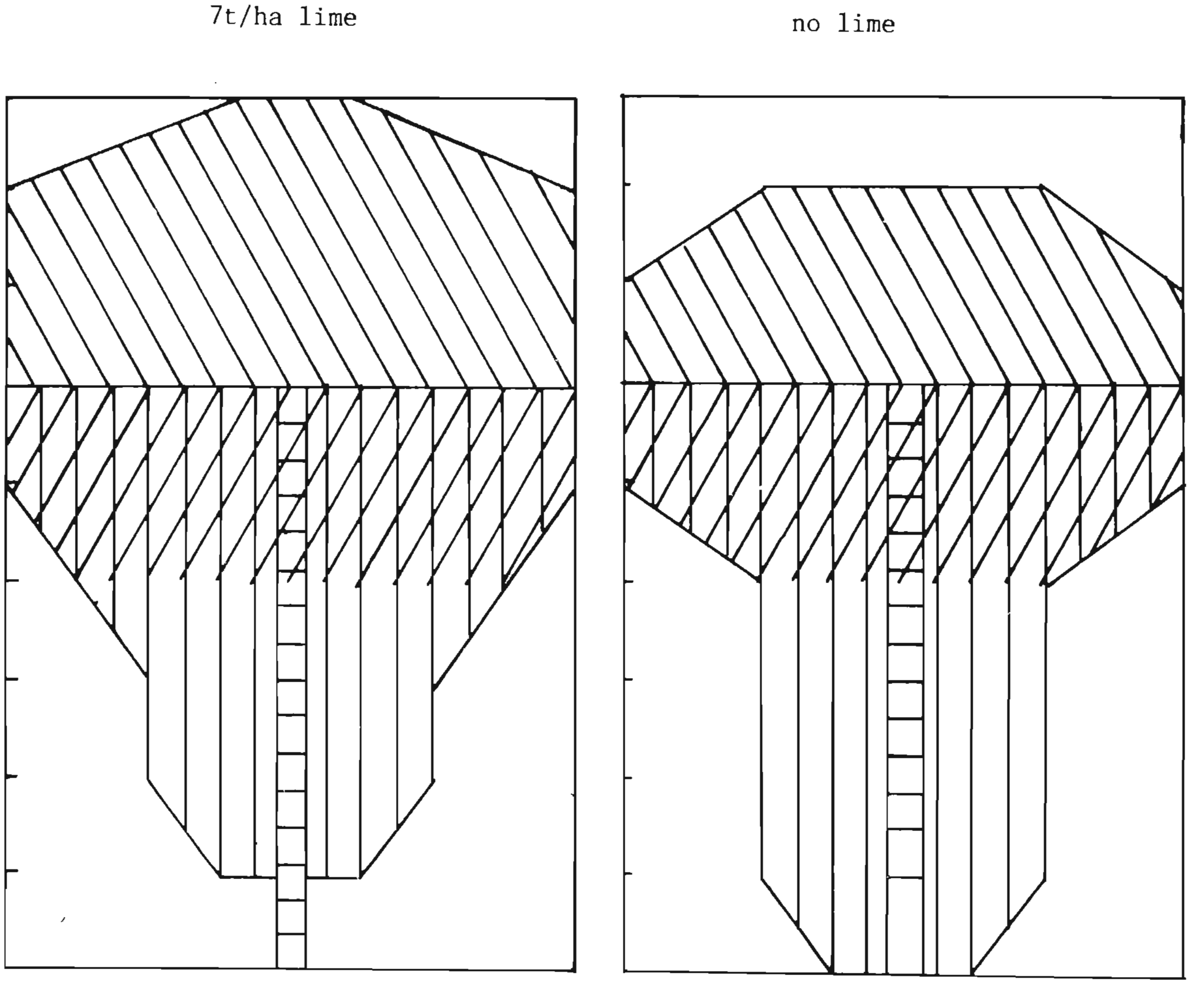

Figure 24. Development of cv. DUSI in acid soils with and without lime application.

From these results it is clear that all the clover cultivars responded positively to lime application and that this resulted in an increased root mass. The increased root formation was, however, limited to the top 20 cm of soil to which the lime was applied. Only in the cultivars LADINO and DUSI was any penetration of the deeper, unlimed, soil observed. In the case of LADINO the roots penetrated to 40 cm while DUSI roots exceeded the sampling depth of 60 cm. The primary taproots of LADINO and DUSI were also strongly developed in both the limed and unlimed situations and were rated as 8 for LADINO and >10 for DUSI on a 1 to 10 scale. PITAU and HUIA formed taproots which were severely stunted but thickened under the no lime situation, while at the time of sampling no taproots were observed in IRRIGATION. The above ground DM production was directly in proportion to the root development observed.

Woodfield and Caradus (1987), working on the adaptation of white clover to moisture stress, found that cv. DUSI had a high proportion of root but a low proportion of taproot. This is a confusing way to describe the situation since in Table 3 of their paper DUSI taproots are reported to be 2,22mm in diameter, i.e. thicker than any other cultivar, selection or ecotype, and to have 6,4 taproots per plant compared to 6,9 for HUIA, 8,2 for the "root knot nematode resistant" selection and 2,6 for a selection "low yields at low P ". This means that for DUSI to have a low proportion of taproot the fibrous root numbers must have been extremely high, resulting in an extensive root system. This finding indicates that DUSI root systems follow the same trend in New Zealand as in South Africa where DUSI was developed. The effectiveness of the root system, however, should be measured by soil profile penetration and therefore mass of fibrous roots is also very important.

In addition rooting depth is of great importance in the RSA as moisture is often confined to the lower soil layers. A tolerance to Al combined with the ability to form taproots is essential, thus indicating that selection should not only be based on one characteristic at a time but that the whole complex influencing pasture production must be taken into account.

CHAPTER 5

ALUMINIUM TOLERANCE IN WHITE CLOVER

5.1 LABORATORY TECHNIQUES FOR THE SELECTION OF WHITE CLOVER PLANTS WITH HIGH ALUMINIUM TOLERANCE

Although in vivo selection of plants for tolerance to high aluminium concentrations formed a part of the DUSI white clover development programme, it was nevertheless regarded as desirable to ascertain whether a laboratory technique could be developed whereby the selection process can be speeded up, particularly so since Dobrenz (pers. comm. 1986 *) has been successful in isolating lucerne plants tolerant to alkaline conditions by repeated selection in the presence of increasing concentrations of salt.

1.1 Materials and Methods

Three replications of 20 seeds each of the white clover line 501 were scarified with fine carborundum paper and placed in petri dishes at 20^{o}C to germinate. The seeds were watered with a solution of deionized water containing Al as aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) at the following concentrations: 0, 150, 300, 450, 600 and 900ppm.

* Dobrenz, A.K., University of Arizona, Tucson, Arizona.

Seed germinating at the different concentrations was transferred to pots in the glasshouse, the plants emanating from seed at each concentration being isolated from the others. Pollination was effected within groups and the seed harvested to form the basis for a similar but expanded three replicate experiment. The whole procedure was repeated a third time.

5.1.2 Results

Germination percentages for the three consecutive treatments are presented in Tables 20, 21 and 22.

Table 20. The percentage germination of *T.repens* seeds (line 501) at different Aluminium concentrations.

	ppm Al						
Replication	0	150	300	450	600	750	900
A	96	48	20	12	8	8	0
B	100	16	12	28	4	0	0
C	88	44	16	16	4	4	0
Seed group	1	2	3	4	5	6	

From Table 20 it is evident that unselected seed of line 501 fails to germinate in solutions containing 900 ppm Al and that a drastic reduction in the number of germinating seeds occurs at increasing Al concentrations.

Table 21.The percentage germination of <u>T.repens</u> seeds from Al selection (cycle 1) at different Aluminium concentrations.

Origin of seed		ppm Al						
Group/Reps		0	150	300	450	600	750	900
	A	72	0	0	0	0	0	0
1	B	64	4	0	8	0	0	0
	C	64	4	4	4	4	0	0
	A	84	32	4	4	0	8	0
2	B	60	8	0	8	4	0	0
	C	64	16	0	0	0	0	0
	A	72	20	12	4	8	4	0
3	B	56	8	12	16	0	4	0
	C	76	16	8	4	0	0	0
	A	44	20	4	4	0	0	0
4	B	52	0	4	4	4	0	4
	C	52	8	8	0	4	0	0
	A	56	4	0	4	0	0	0
5	B	52	8	0	0	0	0	0
	C	52	4	4	4	0	0	0
	A	64	40	0	4	4	0	4
6	B	72	24	4	4	4	4	4
	C	64	24	8	0	0	0	4
Seed group		7	8	9	10	11	12	13

The results in Table 21 indicate that the progeny of the first selection cycle produced seeds that germinated at increasing frequencies at higher concentrations of Aluminium.

Although seed collected from plants that germinated at 750 ppm Al in the first cycle produced seeds that germinated at 900 ppm Al,group 13, these seeds could not be grown to form productive plants because of very severe inhibition of root formation at the high Aluminium level.

Table 22. The percentage germination of T.repens (line 501) seed selections from the second cycle of selection for tolerance to high aluminium concentrations.

Origin of seed		ppm Al						
Group/Reps		0	150	300	450	600	750	900
	A	75	50	45	10	15	5	0
7	B	75	55	45	5	0	5	0
	C	80	45	30	10	5	0	0
	A	75	35	10	10	5	0	0
8	B	75	40	10	0	5	0	0
	C	75	55	10	10	0	0	0
	A	80	20	20	20	0	0	5
9	B	90	40	60	5	5	10	5
	C	95	45	30	15	0	0	0
	A	90	65	30	10	0	0	0
10	B	100	65	25	5	0	10	0
	C	80	80	10	5	0	0	0
	A	85	70	60	5	25	0	0
11	B	70	75	60	5	5	5	0
	C	90	85	20	5	10	0	0
	A	75	65	40	50	55	20	15
12	B	80	55	25	15	50	15	20
	C	65	55	30	10	25	5	10

The results presented in Table 22 on the third selection cycle again indicate that positive selection can be made for Aluminium tolerance. A relative high percentage of germination at 900 ppm Al was found in seeds resulting from the second selection cycle at 750 ppm Al, i.e. group 12. The inclusion of plants that germinated at high levels of Al in the first cycle (groups 5 and 6) into the gene pools that produced seeding groups 7 to 13, did cause an increase in the percentage seeds in groups 7, 8 and 9 that germinated at the higher Al levels.

5.1.3 Discussion

Although it is possible to increase Al tolerance of T.repens through recurrent cycles of germination of seed at relatively high concentrations of Al, the period of time required to produce seed from plants selected by this method is still considerable.

By using techniques like tissue culture and thus speeding up the life (selection) cycle of superior plants tremendous progress should be possible. The method will, however, be of limited use in a breeding programme where simultaneous selection pressures for a range of characteristics are being applied.

Caradus et al (1987) feel that there is little intra specific difference between white clover cultivars as far as germinating in Al concentrations is concerned and find that cv. HUIA has as much Al tolerance as cultivars like DUSI. The trials were, however, done for 30 days and not extended to growing the plants in high Al soils. No indication is given of improvement in Al tolerance due to successive selections at high Al levels.

It is felt that the planting of large populations of plants on soils that are high in Aluminium and where the simultaneous selection for characteristics like grazeability, persistence, root formation, leaf colour, disease resistance etc. can be carried out is still the most viable and by far the most economic method of plant improvement that can be applied. This would specifically apply to improvement of relatively poorly known crops or crops that are poorly adapted to areas in which such limiting factors are prevalent and in which such a crop has to be used.

If, however, a cultivar is acceptable in all respects, but needs improved tolerance to a single factor, one should be able to apply the method for increased tolerance with great success.

CHAPTER 6

VESICULAR ARBUSCULAR MYCORRHIZAS AND WHITE CLOVER

6.1 THE TESTING OF PROGENY FROM THE IMPROVEMENT PROGRAMME FOR THE INFLUENCE OF VESICULAR ARBUSCULAR MYCORRHIZAS ON DRY MATTER PRODUCTION AND NUTRITION

6.1.1Introduction

Plant roots form mycorrhizas with certain fungi, resulting in a mutually beneficial symbiosis. The predominant and most widespread group are the vesicular arbuscular mycorrhizas(VA) in the Endogonaceae (Zygomycetes). These fungi form vesicles (terminal hyphal swellings) and arbuscles (intracellular haustoria) and do not change the root structure (Gerdeman,1975).

According to Mosse(1973) mycorrhizal plants seem to have several advantages over non mycorrhizal plants in infertile soils, largely because of increased uptake of nutrients, such as phosphorus, that are relatively immobile in the soil.The volume of soil permeated by mycorrhizal hyphae is much greater than with plant root hairs. There can be as much as 80 cm hyphae for each 1 cm of root hair.

Unfortunately, VA mycorrhizal fungi are obligate symbionts that must be increased on living plant roots and therefore can not be grown on laboratory culture media for general agricultural use. A method for commercial production of VA mycorrhizal fungi on living plant roots has been developed but has not been widely implemented (Menge, Lembright & Johnson, 1977).

According to Gerdeman (1975) the available evidence suggests that mycorrhizal infection may increase the rate of nitrogen fixation by Rhizobium species in leguminous plants. This is a subject of great importance and deserves further study.

Mosse (1972) has found a certain amount of host specificity in mycorrhizas and also that some VA fungi are more beneficial to their hosts than others.

Crush (1976) indicates that for some legumes like lucerne and alsike clover, endomycorrhizal fungi are likely to be parasitic. Red clover seems to benefit from mycorrhizas at low fertility levels but the upper fertility limits for a beneficial effect for white clover have not been established.

In New Zealand, Powel (1976) has shown that in many hill country soils, white clover is highly dependent on infection by the indigenous mycorrhizal fungi for phosphorus uptake and plant growth. These experiments done in sieved sterilized soils showed that some introduced mycorrhizal fungi were even more efficient in stimulating P uptake than the indigenous fungi. This stimulation by the fungus E_3 was found to be 90% greater shoot growth from white clover in Te Kuiti soil and a 50% higher P uptake in a Mahoenui soil. E_3 stimulated a 42% and 32% higher P uptake than indigenous fungi at 0 and 11,2 kg P/ha respectively but 108% higher P uptake at 44,8 kg P/ha, making it well suited to use in higher fertility situations (Powel,1977).

To determine the distribution of VA mycorrhizas in Natal pastures and the influence of such mycorrhizas on the nutrition of white clover, especially P nutrition, which is the single most limiting factor influencing clover growth in the high potential areas, a survey was conducted. This indicated a very wide distribution of VA mycorrhizas in Natal pastures, (Pasture survey 1978/79 unpublished data). Consequently it was decided to establish a trial to determine the effect of different VA mycorrhizas on white clover growth.

6.1.2 Materials and Methods

On the basis of the survey mycorrhizas were collected from the clover growing areas of Natal and together with two imported mycorrhiza lines an observation trial was conducted using sterile soils. The two imported mycorrhiza lines were *Glomus tenuis* and E_3 (an isolate of *Glomus fasciculatus*). They were obtained from Dr. C. Ll. Powel of Ruakura Research Station, New Zealand. Mosse (1975) has shown that for several legumes E_3 is more efficient at stimulating P uptake, plant growth and nodulation than the indigenous mycorrhizal fungi in many soils from Britain and

Africa. She found that E_3 was preferred and propagated by the legume roots in these soils at the expense of the less efficient indigenous fungi.

From the observation trial the two lines E_3 and G.tenuis were shown to be superior and inoculae were cultured. A field experiment was conducted to determine the effect of these mycorrhiza lines on the growth of white clover line 501, as well as their competitiveness with indigenous lines.

The experiment was established at Cedara, on a well drained soil of the Hutton series. The soil pH was 4,2 in KCl and P content was raised to 20 ppm according to the 0,01N H_2SO_4 ,P test.

Seeds of Trifolium repens line 501 were sown after inoculation with commercial rhizobia and pelleting in lime with MoO_3 added at 300g/ha. Two lines of mycorrhiza were introduced to the plots as root cultured inoculae. To ascertain the competitive ability of introduced mycorrhizal fungi and their relative benefit to the clover as compared to those of indigenous fungi the treatments were duplicated on sterilized field plots. Basamid was applied to those plots that were to be sterilized at the recommended rate. The experiment was established on 18 March 1980. The first harvest was taken on 18 August 1980 and thereafter at three week intervals until 18 May 1981, to give a total of 14 cuts.

The DM production of white clover under irrigation, as well as P, crude fibre (CF) and crude protein (CP) content of clover tops over the season was compared following the inoculation with the VA endophytes.

The field experiment was laid out as a five replicate randomised blocks design with the following treatments:

T.repens on unsterilized soil with E_3; *T.repens* on sterilized soil with E_3; *T.repens* on unsterilized soil with *Glomus tenuis*; *T.repens* on sterilized soil with *G.tenuis*; *T.repens* on sterilized soil and *T.repens* on unsterilized soil.

Samples for eelworm were taken from the test site before establishment and no eelworm deleterious to white clover was found.

A mycorrhizal survey showed numerous spores of unidentified VA mycorrhizal species present in the soil. Plants from the different treatments were inspected regularly for nodulation and mycorrhizal infection.

6.1.3 Results

Effects of sterilization

Sterilizing the soil with Basamid not only killed the micro organisms, but the plots were also weed free after the treatment.

Nodulation

Ten white clover seedlings were removed at random from each plot 10 weeks after planting and examined for nodulation. All the plants were well nodulated and showed healthy pink nodules. A slightly reduced number of nodules was observed on plants from sterilized plots to which no mycorrhizas had been added.

Mycorrhiza infection

Concurrently with the initial examination for nodulation, and on three more occasions during the growing season plant roots were examined for the presence of VA mycorrhizas. All the plants from unsterilized plots as well as plants from sterilized plots inoculated with VA mycorrhizas showed infection from week 10 onwards. Plants sampled from sterilized, non inoculated plots started to show traces of infection at 40 weeks after planting and at the end of the season only a very light mycorrhizal infection was observed compared to the dense masses of hyphae found on the roots of inoculated plants and plants exposed to naturally occurring mycorrhizas.

White clover growth and dry matter production

During the seedling stages it appeared that growth was slightly depressed in the sterilized plots. At first harvest 22 weeks after planting there was, however, no significant yield depression.

The growth pattern of white clover as reflected by growth curves of the different treatments (Fig.25) did not vary significantly and growth rates reached their peaks at the same time during the growing season (Fig.26).

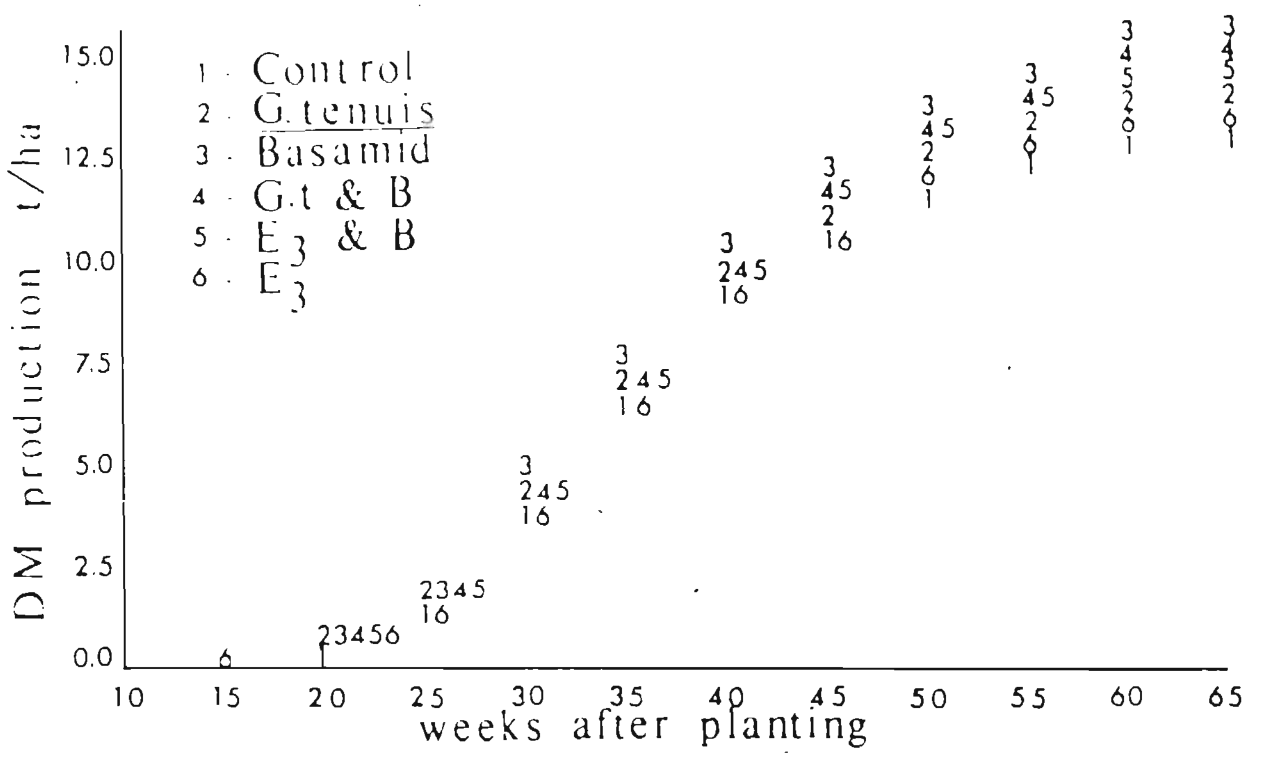

Figure 25. Dry matter production of T. repens at Cedara.

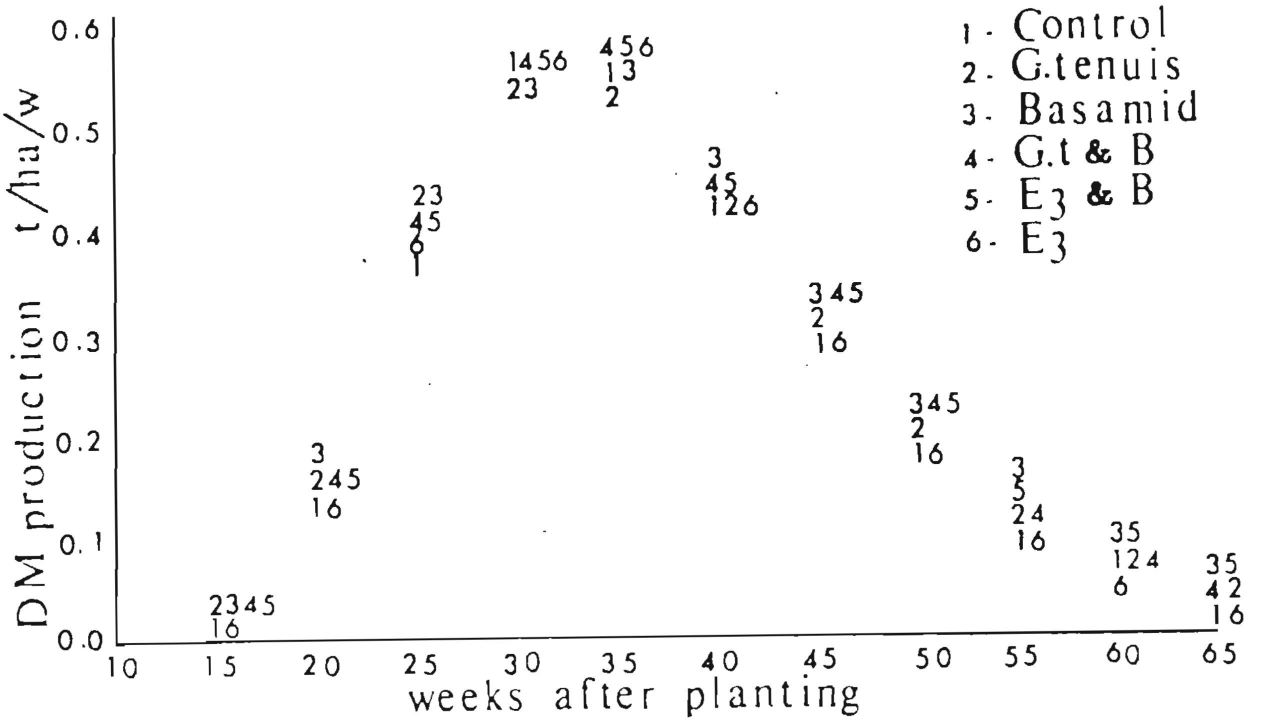

Figure 26. Dry matter production rate of <u>T. repens</u> at Cedara.

Inoculation of the clover with introduced mycorrhizas did not have a significant influence on total DM yields obtained during the year, but sterilizing the soil did have a beneficial effect on total DM yield (Table 23).

Table 23. Dry matter yields of white clover line 501 inoculated with mycorrhiza strains at Cedara.

Treatment	Herbage yield (t DM/ha)
Basamid	14,98
Basamid + G.tenuis	14,88
Basamid + E_3	14,55
G.tenuis	13,93
E_3	13,55
Control	13,36
LSD (p=0,05)	0,74
LSD (p=0,01)	1,10
cv	3,94%

Phosphorus

Phosphorus content of plant tops was monitored throughout the growing season and there seemed to be a general trend of lowering of the P content as the season progressed. There were, however, no significantly higher P levels in mycorrhizal plants than in non mycorrhizal plants towards the end of the season.

Nitrogen

Nitrogen content of the plants, or effective nodulation, was the only aspect monitored that showed a benefit from mycorrhizas, whether introduced or indigenous.The plants grown in sterilized plots to which no mycorrhizas had been added showed a marked reduction in CP content throughout the growing season(Table 24).

Table 24. Crude protein content of white clover line 501 tops over the 1980/81 season at Cedara.

Treatment	% CP on		
	80/09/30	80/12/12	81/03/16
Basamid + E_3	25,47	27,01	24,60
<u>G.tenuis</u>	25,66	29,43	24,77
Basamid+<u>G.tenuis</u>	26,78	26,03	23,83
Control	26,52	24,91	24,61
E_3	26,71	25,61	24,88
Basamid	24,24	23,95	23,34
LSD (p=0,05)			2,04
LSD (p=0,01)			2,90
CV			3,93%

6.1.4 Discussion

The inoculation of plants with mycorrhizal fungi is a relatively new concept in pastoral agriculture. The chief advocates of mycorrhizal inoculation seem to have done most of their experiments in sterilized pot trials. Such experiments often have very little bearing on actual field conditions.
As can be seen from the results of a field trial with both sterilized and unsterilized plots white clover DM production does benefit from inoculation with VA mycorrhizas, but only to a limited extent, 0,2 to 0,5t/ha DM in unsterilized soils. Sterilizing the soil, however, did increase the total DM production markedly but the cost of sterilizing is prohibitive.

Although soil sterilizing improved the DM yield of white clover by 1,6t/ha, the effect of mycorrhizas,and here the indigenous mycorrhizas are included, on N fixation and therefore CP content is of sufficient magnitude to be regarded as of greater importance. Benefit derived from inoculating with mycorrhizas for increased P uptake was negligible in this experiment.

The relatively poor results obtained with introduced mycorrhizas seems to indicate that either the indigenous mycorrhizas are relatively effective or that the competition between indigenous and introduced strains is so strong that the full potential benefit from these strains is not realized.

Benefit derived from the mycorrhizas, however, was greater from *G.tenuis* than from E_3, indicating that this line may be better suited to Natal conditions.

CHAPTER 7

DEVELOPMENT OF SEED PRODUCTION TECHNIQUES

7.1 SEED PRODUCTION REQUIREMENTS

Due to the exclusive use of overseas clover cultivars in the establishment of pastures in South Africa, no seed of white clover is currently being produced in this country, so that approximately 50 000 kg of clover seed has to be imported annually at a loss of foreign exchange exceeding R 300 000, and a total dependence on foreign suppliers. Not only is it important to have a local source of seed, but with ever increasing energy costs, it is becoming more important to have a cheap source of nitrogen for low cost pastures. The increased area that could be established to white clover due to the development of cv. DUSI, would also directly influence the demand for clover seed, and particularly for that of the local cultivar. For the successful implementation of the locally bred cultivar it was also essential to establish guidelines for economic seed production,since these may differ considerably from those applied by the specialist seed producers in Europe and New Zealand. Therefore a seed production strategy, adapted to South African conditions, had to be developed.

This strategy had to conform to the UPOV (International Union for the Protection of new Varieties of Plants) scheme and had to, preferably, include some way of utilizing the clover as a pasture at those times that seed was not being produced. By using the seed production unit as a grazing area the relatively high costs related to pure seed production could be offset against the value of the pasture for production of animal products. The release of a white clover cultivar would thus not only provide South Africa with a better adapted pasture legume but would also stimulate a whole new seed production enterprise.

In view of the above it was decided to conduct a number of experiments to determine the management requirements for the production of *Trifolium repens* seed under South African conditions. The results of these are presented after a review of the relevant literature.

7.2 LITERATURE REVIEW

7.2.1 Factors that affect seed production.

The seed production potential of white clover is determined by a combination of factors, namely the number of inflorescences per unit area, the number of flowers per inflorescence, the number of potential seeds per flower and the viability of the pollen and the embryo (Thomas,1961). Maximum seed production is attained under conditions that ensure the maximum rate of shoot formation and at which the number of nodes that form flower primordia reach a maximum.According to Brougham (1962), high temperatures and long days encourage the formation of nodes and inflorescences.

The tempo of leaf formation seems to differ little for the different white clover cultivars,(Thomas, 1961). Even cultivars that morphologically differ as much as G HUIA, the winter producer TAMAR and the summer growing KENT WILD WHITE are similar. The conclusion is reached that seed production is influenced strongly by temperature and daylength, but little by genotype. It is, however, a known fact that the LADINO type cultivars (large-leaved types) flower much less profusely than cultivars with smaller leaves.

In plants that are in the vegetative stage, the axillary bud only develops in the axil of the third leaf primordium. However, when these plants reach the reproductive stage, the development of the floral buds takes place in the axil of the youngest leaf primordium. Any damage to the growing tips of runners at this stage can result in the reduction of the number of inflorescenses. The change from vegetative to reproductive stage is controlled by daylength and temperature. The maximum flowering period for LADINO types is induced by long days and high temperatures (Thomas,1981). Should flowering be reduced after a prolonged period of long days, it can usually be reinstated by a cutting treatment. This indicates that several consecutive harvests should be possible during a favorable season if seed production takes place under irrigation. There are also strong indications that a deficiency in mineral nutrients may result in depressed seed yields.

On the basis of the low rates of leaf emergence during winter (on average 0,45 leaves per week) it is possible that inflorescences initiated on 1 June, do not appear before the middle of September. This means that the first flush of spring flowers could already be induced during the winter.

White clover is an unusual pasture plant in that no reduction in leaf size takes place when inflorescenses are being produced. Under normal circumstances the only effect of flower production that can be observed is the reduction in the formation of vegetative axillary buds as the inflorescences replace the vegetative buds.

Gibson and Hollowell (1966) and Gibson et al (1971) proved that the longevity of Ladino type clovers is related to flowering intensity and to the duration of the flowering period as relatively few vegetative shoots can be produced in profusely flowering plants during the flowering period.

7.2.2 Management practices that influence seed production

Continuous vegetative development of plants produces an increasing number of nodes from which side shoots and thus inflorescences can be formed. Inflorescence density is the main component of seed yield and is linked to the rate of flower production during the main flowering period. It follows that the main purpose of management for seed production is to ensure maximum inflorescence formation in the shortest possible period to facilitate high seed yields and low seed losses during harvesting.

In imposing a rest period for seed production account must be taken of the very rapid growth that occurs after removal of livestock from the pasture and that this rapid growth lasts for a maximum period of 5 weeks. Should only one seed harvest be required the optimum closing down date for seed production would appear to be the middle of November. With most management practices the main flowering period of white clover seems to be during December, so that seed should be harvested about one month after the middle December.

Another factor that appears to play a significant role in seed production is seeding rate. Clifford (1977) claims that increasing the seeding rate from 3 to 6 kg/ha increased seed production by 16%, but that further increases had no significant effect. It was, however , found that increasing the row spacing from 15cm to 30 cm and 45 cm increased the seed yields by up to 65%.

Clifford (1979, 1986), also stresses that if seed is harvested by combine harvester, care should be taken that sufficient vegetative material is present to ensure the successful functioning of the machine. Should soil moisture be a limiting factor it is advisable rather to remove the grazing animals from the field at an earlier stage to allow an increase in the amount of vegetative material, than to defer the harvesting,this so because a delay of one month in harvesting will normally cause a drastic reduction in seed yield. The late removal of livestock from seed fields also has the effect that a great number of seed heads are borne on very short peduncles, making it impossible to harvest them mechanically.

Preliminary indications are that approximately 33% of the seed produced is lost during the harvesting process, (Clifford,1986), and according to Suckling and Charlton (1978), it may, under New Zealand conditions, amount to as much as 200 kg seed/ha. This seed may lie dormant for years before germinating and may cause major problems in the contamination of subsequent certified seed crops.

To overcome the problem, the New Zealand Agricultural Engineering Institute in conjunction with a large seed firm, Wrightsons, have developed a machine that ensures clean stands of clover by precision spraying of herbicides. The effect of this spraying programme is to reduce contamination of seed production areas by volunteer clovers to less than 1%, which is an acceptable level for the production of certified seed. Although weedicides normally tend to reduce seed yields, this machine by strategic placement of the herbicide prevents such a reduction. It can also be used on already established fields as it is fitted with coulters that cut through the runners and thus ensures that weedicides are not translocated to the mother plants through the sprayed runners.

According to Hyde, Mc Leavy,& Harris, (1959) white clover seed becomes viable about 12 days after pollination while 18 days after pollination the percentage viable seed is as high as 95%. Hardseededness is initiated at about 18 days after pollination, after which it increases rapidly and at 25 days after pollination can be as high as 75%. It is therefore obvious that the stage at which the harvesting of white clover seed takes place can influence both the harvest as well as the quality.

7.2.3 Pollinators

Although Anon.(1974), indicates that the need for pollinators is approximately 1 hive of honey bees per 3ha, indications are that this is not sufficient in warmer countries. During a study tour of the United States of America undertaken in 1986, the writer was informed that for lucerne seed production the optimum number of hives per ha is between 10 and 12, (Roth,1986 pers. comm.*).

* Roth, J., W-L Research Inc., Bakersfield, Ca., USA

It would therefore seem that a greater density of pollinators should be aimed at. Furthermore, studies conducted in California pointed to the importance of a water supply for pollinators close to, or in, the fields used for seed production.

7.3 SEED PRODUCTION EXPERIMENTS AT CEDARA

7.3.1 Materials and Methods

Two experiments for the determination of white clover seed production were established in February 1982 and March 1983.

The experiments were conducted at Cedara on an acid soil of the Hutton form with a pH of 4,1 (KCl). The annual rainfall is 879 mm and frosts occur regularly during winter. The area is described by Phillips (1973) as Bioclimatic subregion 3c.

Fertilizer was applied according to soil analyses and P and K levels were raised to 20 and 120 ppm respectively. No lime was applied to the area and molybdenum was added to the pelleting material at a rate of 300g of MoO_3 /ha.

The white clover was established at a seeding rate of 6 kg/ha at a spacing of 15 and 45 cm between the rows. The experiment was irrigated and cut on a three weekly cycle. The seed used was that of a local white clover selection from the breeding programme for cv. DUSI.

Closing down dates were 15 October, 15 November and 15 December, on which dates the plots were cut and then left to set seed.

Seed was harvested at two and three months after closing down and seed quality was monitored with respect to germination percentage and hardseededness. Hard seed is defined as those viable seeds that do not germinate on wet filter paper after a period of 8 days at a temperature of 18^{o} C.

Unfortunately no estimates could be made of the amount of seed not recovered during harvesting either as a result of shattering or due to non harvest of inflorescences with short peduncles.

The experimental layout was a fully randomised factorial design with three factors and three replications. The factors were 3 closing down dates, 2 harvest dates and 2 row spacings.

7.3.2 Results

Forage was harvested throughout the growing season at three weekly intervals until the designated closing down dates. Thereafter the plots were left for two or three months according to the experimental procedure, a seed harvest was taken, and the plots were again included in the three weekly forage harvest cycle. The results presented in Tables 25, 26, and 27, give an indication as to relative seed production for the different treatments, seed germination percentages and DM yields.

Table 25. The relationship between close down date, close down period , yields of dry matter and seed and seed quality for white clover grown at Cedara in the Natal mistbelt during the 1982/83 season,(first season).

CLOSE DOWN DATE	YIELD IN KG/HA	CLOSE DOWN PERIOD (MONTHS) 2		3	
		ROW SPACING (CM)			
		15	45	15	45
	DM	7210	5750	6560	5250
15/10	SEED	--	--	56,1	44,8
	% G SCAR			51	54

G = germination   SCAR = scarified seed

Table 25. (cont.)

CLOSE DOWN DATE	YIELD IN KG/HA	CLOSE DOWN PERIOD (MONTHS) 2		3	
		ROW SPACING (CM)			
		15	45	15	45
15/11	DM	7740	6850	7730	6880
	SEED	42,7	102,4	14,3	28,3
	% G SCAR	55	56	84	77
15/12	DM	8990	7390	8250	6830
	SEED	21,6	24,4	--	4,5
	% G SCAR	80	83	--	79

Table 26. The relationship between close down date, close down period , yields of dry matter and seed and seed quality for white clover grown at Cedara in the Natal mistbelt during the 1983/84 season (second season).

CLOSE DOWN DATE	YIELD IN KG/HA	CLOSE DOWN PERIOD (MONTHS) 2		3	
		ROW SPACING (CM)			
		15	45	15	45
15/10	DM	2761	3112	3510	3919
	SEED	16,95	17,77	8,96	8,09
	% G SCAR	80	66	73	78
	% G UN SC	32	27	30	33

UN SC = un-scarified seed

Table 26. (cont.)

CLOSE DOWN DATE		CLOSE DOWN PERIOD (MONTHS)			
		2		3	
	YIELD IN KG/HA	ROW SPACING (CM)			
		15	45	15	45
15/11	DM	4713	4997	4157	5112
	SEED	13,93	18,08	3,28	8,29
	% G SCAR	70	63	67	75
	% G UN SC	42	34	13	18
15/12	DM	5448	6176	5975	5136
	SEED	7,10	10,10	8,96	13,15
	% G SCAR	64	77	84	74
	% G UN SC	20	25	17	13

Table 27. The relationship between close down date, close down period , yields of dry matter and seed and seed quality for white clover grown at Cedara in the Natal mistbelt during the 1983/84 season,(first season).

CLOSE DOWN DATE		CLOSE DOWN PERIOD (MONTHS)			
		2		3	
	YIELD IN KG/HA	ROW SPACING (CM)			
		15	45	15	45
15/10	DM	5991	5557	5986	5955
	SEED	6,94	10,19	5,59	8,85
	% G SCAR	75	73	70	87
	% G UN SC	21	14	14	18

Table 27.(cont.)

CLOSE DOWN DATE	YIELD IN KG/HA	CLOSE DOWN PERIOD (MONTHS) 2		3	
		ROW SPACING (CM)			
		15	45	15	45
15/11	DM	8112	7223	7763	5298
	SEED	7,65	8,33	20,76	55,49
	% G SCAR	77	74	85	88
	% G UN SC	17	14	8	13
15/12	DM	5673	5517	6409	4455
	SEED	39,90	56,73	--	--
	% G SCAR	89	92	--	--
	% G UN SC	9	10	--	--

The results indicate that seed yields are consistently higher at the wide row spacing of 45 cm than at 15cm. Furthermore a two month close down period results in more seed being harvested than a three month period, a notable exception being the November close-down in 1983/84 (Table 27).

It can be reasoned that it could be advantageous to close down on 15 October for two months, harvest seed on 15 December and close down again for a second seed harvest in February thus taking two seed harvests instead of the normal one.

The germination results show that a high percentage of hard seededness occurs in cv. DUSI. It is therefore necessary to stress the need for seed scarification when germination tests are being conducted.

For the establishment of spring pastures it would also be advisable to scarify the seed, but should dryland pastures be established, such scarification could be a disadvantage as the delayed germination of a percentage of the seeds, until sufficient soil moisture for successful germination is present, could ensure the ultimate success of pasture establishment.

Figure 27. A registered breeder seed production unit of T. repens cv. DUSI established at Cedara in March 1984, (32 months previous to the date on which this photograph was taken.

A registered breeder seed production unit which was established at Cedara in March 1984 using 200 g of DUSI seed per ha, established slowly due to dormant weeds which dominated the area at the low clover seeding rate (normal 3 kg/ha). With the use of herbicides the weeds were, however, controlled during the second season and a good stand of DUSI was established, as illustrated in Fig. 27.

An inspection during October 1986, 32 months after establishment showed no thinning of the stand due to the "30 month syndrome" and active colonization of the few remaining bare patches in the pasture was taking place.

CHAPTER 8

REGISTRATION OF THE CULTIVAR

Breeding and evaluation of a pasture plant that will be accepted by the farming community as able to make a positive contribution to profitable farming practices is only half the problem confronting the breeder. Seed of this cultivar must thereafter be produced and made available to the farmers. For this, not only need seed production techniques be developed, but it is also essential to ensure that the quality of the seed and genetic purity is of a high standard and true to type.

It is therefore necessary to register the cultivar with the Directorate of Plant and Seed Control in South Africa and eventually with the OECD. Plant breeders rights can also be obtained and a specification that only certified seed may be sold can be included in the registration procedure.

This will ensure that farmers buy seed that is only two generations removed from breeders seed. To ensure that the genetic purity of cv. DUSI can easily be monitored a recessive gene, no leaf marking, was introduced into the cultivar.

8.1 TECHNICAL DESCRIPTION OF *T. REPENS* CV. DUSI

1. Species: *Trifolium repens* L.

2. Breeders reference number: Tr 508

3. Proposed denomination: *Trifolium repens* cv DUSI

4. Information on the origin, maintenance and reproduction of the variety as well as full details of composition: DUSI white clover is a synthetic derived from selection over a 6 year period.The original source material consisted of numerous imported an locally collected lines. Thirty eight selected mother lines are being maintained in an open pollinated block for the production of pre breeders seed.

5. Characteristics of the variety to be indicated:

Characteristic		Example variety	
5.1 Leaf: length of central leaflet at time of flowering; 3rd to 4th leaf from end of tip of rapidly growing stolon	very short	KENT WILD WHITE	[ ]
	short	S 184	[ ]
	medium	MILKANOVA	[ ]
	long	GIGANT	[X]
	very long	LADINO	[X]

5.2 Leaf: width of central leaflet (as for 5.1)	very narrow	KENT WILD WHITE	[ ]
	narrow	S 184	[ ]
	medium	MILKANOVA	[ ]
	broad	GIGANT	[X]
	very broad	LADINO	[X]
5.3 Leaf: mark	absent	PRONITRO, GIGANT	[X]
	present	TAMAR	[ ]
5.4 Leaf: length of petiole (as for 5.1)	short	S 184	[ ]
	medium	MILKANOVA	[ ]
	long	GIGANT	[X]
5.5 Time of flowering (in the year after sowing when three florets per plant are open)	very early		[ ]
	early	VON KAMEKAS	[ ]
	medium	HUIA, S 184	[X]
	late	GIGANT	[ ]
	very late		[ ]

6. Similar varieties and differences from these varieties:
The names of already recognized varieties of the same kind with which DUSI is comparable are LADINO and HAIFA.

It is internationally accepted that the number of secondary tap roots is directly correlated with leaf size and that large-leaved white clover types have more secondary taproots than small-leaved types. DUSI, however, produces more secondary taproots than LADINO or HAIFA. Although this is difficult to quantify and could differ according to cultivation practices the presence of secondary taproots is important.

The leaves are characteristically large like LADINO but smaller than those of TAMAR. Leaf colour is green like LADINO and not yellow/green like HAIFA. The leaf marking so prevalent in HAIFA and LADINO is all but absent in DUSI and should not occur in more than 5% of the population.

Stolons are thick and strongly perennial, often slightly stunted under dryland conditions with strong secondary taproots developing from the nodes.

The main flowering period is November to December and flowers are white to pinkish white. Seeds show a degree of hard seededness especially when left to ripen on the plant.

DUSI white clover was bred for its suitability to cultivation in the high potential areas of South Africa. It produces as well as LADINO under irrigation and normally better than LADINO under dryland conditions.

Because of its specific adaptation to adverse growing conditions DUSI white clover is purposely not uniform. Uniformity does, however, exist for:

1. The production of secondary taproots.
2. Large to medium large leaves
3. Less than 5% of the population has a white leaf mark.

7. Special requirements for conducting tests:
   Seed to be scarified.

8. Place and season in which characteristics in section 5. were noted: Cedara 1983/84.

8.2 REGISTRATION OF THE CULTIVAR

The *T.repens* cv. DUSI was inscribed on the South African Variety List (held in accordance to Article 15 of the Plant Improvement Act,1976), in April 1986.

A Certificate of Grant of Plant Breeders Right, (according to the Plant Breeders Rights Act, 1976, Act 15 of 1976), was issued on 13 August 1986, and a registration number ZA 86289 awarded.

Plant Breeders Rights were applied for in the name of the Executive Director of the Department of Agriculture and Water Supply.

CHAPTER 9

THE PRESENT STATE OF CV. DUSI

At the end of 1986 a small quantity of Breeders seed of cv DUSI was released to SAFSA (South African Forage Seed Association) for distribution to interested seed producers. Seed production under South African conditions was not very successful and only approximately 20 kg of basic seed was produced. Breeders seed was, however, produced at Cedara and this again was made available to SAFSA. During 1987 negotiations were entered into for the production of certified seed in New Zealand and the USA and seed was supplied to the relevant companies.

It is felt that the acceptance of any pasture legume by the farming community is directly coupled to the availability of large quantities of reasonably priced seed, and therefore it is essential that seed be made available, whether of local or overseas origin. To ensure genetic stability of the cultivar, seed will only be produced under strict certification measures, from breeders seed supplied ex Cedara.

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ADDENDUM 1.

CLIMATIC CONDITIONS AT CEDARA AGRICULTURAL RESEARCH STATION

Latitude: 29^{o} 32' N

Longitude: 30^{o} 17' E

Altitude: 1067 m

Long term summary of climatic parameters:

Maximum temperature, ^{o} C

Jan	Feb	Mar	Apr	May	June
25,0	24,9	24,2	22,5	20,6	19,0

Jul	Aug	Sep	Oct	Nov	Dec
19,2	20,8	22,3	22,6	23,5	24,7

Minimum temperature, ^{o} C

Jan	Feb	Mar	Apr	May	Jun
14,6	14,6	13,5	10,5	6,6	3,7

Jul	Aug	Sep	Oct	Nov	Dec
3,7	5,8	8,5	10,6	12,1	13,6

Rainfall, mm

Jan	Feb	Mar	Apr	May	Jun
137,9	127,2	112,6	54,4	30,1	15,1

Jul	Aug	Sep	Oct	Nov	Dec
16,1	23,0	42,3	85,9	111,1	129,3

Evaporation, mm

Jan	Feb	Mar	Apr	May	Jun
151,2	131,6	134,0	101,5	93,1	81,1

Jul	Aug	Sep	Oct	Nov	Dec
94,3	121,2	134,9	142,4	133,0	159,8

Grass minimum temperature °C

Jan	Feb	Mar	Apr	May	Jun
12,6	12,1	10,8	6,9	1,7	-1,9

Jul	Aug	Sept	Oct	Nov	Dec
-2,0	0,9	4,9	8,1	10,0	11,4

ADDENDUM 2.

YEARLY SUMMARY OF RAINFALL (mm) FOR THE PERIOD 1976 TO 1985.

Year	Jan	Feb	Mar	Apr	May	Jun
1976	208,1	149,2	244,3	61,7	34,9	0,5
1977	131,0	25,0	128,6	31,8	21,7	9,5
1978	149,5	81,8	86,6	53,5	5,5	0,7
1979	90,5	108,1	106,9	45,7	53,7	1,7
1980	108,9	62,5	38,6	18,3	14,7	0,0
1981	106,1	91,5	42,2	31,3	36,0	30,5
1982	83,7	86,3	183,1	21,9	7,7	9,8
1983	74,5	24,4	96,9	32,0	25,9	7,5
1984	151,7	77,2	82,7	80,7	4,0	23,2
1985	149,9	290,4	87,7	5,8	3,0	0,1

	Jul	Aug	Sep	Oct	Nov	Dec
1976	2,2	25,0	56,0	145,1	80,1	125,5
1977	0,3	20,8	50,9	87,5	80,0	173,2
1978	1,9	17,8	77,6	138,3	122,2	168,9
1979	29,4	42,1	33,1	65,7	80,0	114,4
1980	2,3	9,5	97,1	34,2	109,4	71,8
1981	3,5	61,0	53,6	27,3	163,8	50,4
1982	3,3	4,0	45,4	96,5	76,0	93,8
1983	33,7	30,7	22,9	66,1	209,9	114,1
1984	10,4	37,0	22,4	121,0	54,3	103,8
1985	5,8	0,7	49,1	162,5	107,6	142,9

ADDENDUM 3.

YEARLY SUMMARY OF EVAPORATION (mm) FOR THE PERIOD 1976 TO 1985

Year	Jan	Feb	Mar	Apr	May	Jun
1976	114,3	120,4	115,6	94,4	75,8	72,6
1977	146,3	143,4	98,3	111,1	92,7	81,4
1978	121,5	110,0	118,0	87,7	90,0	71,4
1979	155,5	124,1	122,0	98,7	76,1	84,2
1980	137,6	150,0	135,1	121,8	111,7	93,0
1981	149,6	114,2	134,7	129,3	82,1	80,0
1982	131,3	145,3	122,7	91,4	90,6	78,3
1983	150,0	146,9	156,9	125,5	118,4	85,5
1984	151,8	129,8	101,9	119,1	98,0	75,7
1985	173,4	122,0	135,2	129,3	95,5	86,1

	Jul	Aug	Sep	Oct	Nov	Dec
1976	94,1	103,2	135,2	108,1	155,9	159,3
1977	99,5	115,2	138,4	120,9	137,4	169,6
1978	84,7	90,7	116,8	123,6	110,9	148,8
1979	83,4	91,2	95,4	123,8	127,7	142,9
1980	100,3	125,2	95,8	133,9	143,8	168,3
1981	92,0	111,5	119,6	151,8	158,8	144,1
1982	92,8	121,0	126,4	143,3	141,1	165,6
1983	100,8	115,2	175,4	124,2	114,3	156,1
1984	83,8	113,5	132,6	136,1	131,8	171,0
1985	114,3	162,7	148,6	160,1	160,3	156,4