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THE ECOLOGY AND USE OF SUBTERRANEAN CLOVER *(TRIFOLIUM SUBTERRANEUM h.)* AS A PASTURE LEGUME. AN AUSTRALASIAN PERSPECTIVE

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SUMMARY

Australian and New Zealand research investigating the ecology and use of the annual pasture legume subterranean clover *(Trifolium subte rraneum L.)* is reviewed. The species and its distribution is described, together with the soil conditions, acidity and fertility to which it is adapted. Vegetative responses to light and temperature are briefly considered. Environmental control of reproduction is described in relation to vernalisation; seed dormancy; hardseed development and decline; and burr burial.

The history and manner of use of subterranean clover in agriculture is detailed. The importance of seed production for agricultural success is discussed, and the effect of grazing on seed production is considered. Problems associated with hardseededness are listed together with possible solutions. The usefulness of hard seed carryover is debated. Symptoms associated with the occurrence of oestrogenic substances in this species are detailed. The methodology of choice of a variety for a given locality is illustrated. Management is described to ensure good initial establishment, and to assist recurring autumn re-establishment. Use of the species as a puré sward or in mixture with grass is debated. Herbage productivity obtained in various localities is catalogued, and quality for animal performance is discussed. Pests and diseases are catalogued, together with an assessment of relative importance. Future directions for research are suggested.

Key words: Management, seed reserves, hardseed.

DESCRIPTION

Subterranean clover is an annual legume divided into several, but mainly three subspecies- *Trifolium subterraneum* L. subsp. *subterraneum,* var *subterraneum;* T. *subterraneum* L. subsp. *brachycalycinum* (Katz. *et* Morley) var *brachycalycinum;* and T. *subterraneum* L.. var *yanninicum* (Katz. *et* Morley) Zohary and Heller- differentiated mainly (Katznelson, 1974; *in* Rossiter, 1978) but not entirely (Zohary and Heller, 1984) on the basis of edaphic adaptation.

Flowering occurs early in spring so that the plant can mature seed before moisture stress curtails plant growth. The species exists over the dry part of the year as seed , and germinates with the advent of rain in the autumn. After germination in the late autumn the seedling rapidly grows a taproot, to be followed in midwinter by from one to twenty shoots (runners) depending on competition, which closely hug the ground and spread out from the crown in all directions. With an increase in daylength and temperature, from three to five florets appear on the peduncle in each leaf axil (Figure 1). Normally one or two fertilised ovules develop in each inflorescence. On fertilisation the florets reflex on the peduncle, and at the same time sterile florets develop (Figure 1) into 30 to 40 hooked

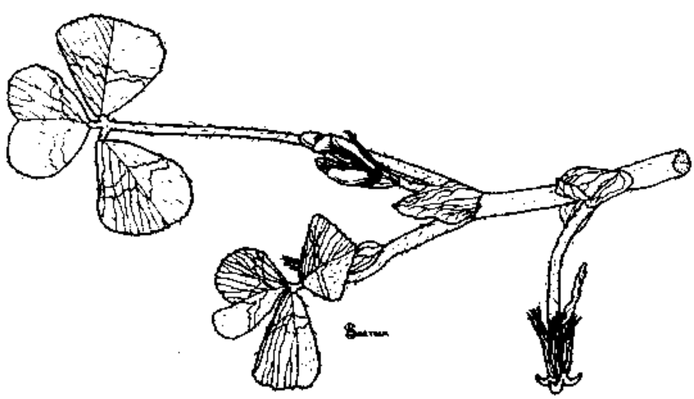

FIGURE 1

A runner of subterranean clover *(Trifolium subterraneum* L.) with a newly formed inflorescence at the centre, and on the right, one where the fertilised florets have reflexed upon the peduncle, which is growing downward to the ground surface. The start of growth of some of the sterile flower parts can be seen below the base of the reflexed florets.

Tallo rastrero de trébol subterráneo (Trifolium subterraneum L.). En el centro: una inflorescencia recién formada. A la derecha: inflorescencia cuyas flores fecundadas se han replegado sobre el pedúnculo, que crece hacia la superficie del suelo. El comienzo del crecimiento de algunas partes estériles de la flor puede verse debajo de las flores replegadas.

structures which envelope the developing seeds to form the "burr" (Figure 2). During this period the peduncle exhibits negative geotropism and grows towards the ground, and a proportion of the burrs become buried. This is also facilitated by the fact that each time the hook structures absorb moisture they expand, whereas when they dry they contract,

FIGURE 2

A ripe burr of subterranean clover *{Trifolium subterraneum* L.), containing two seeds enclosed in the remains of the flower parts, and surrounded by many wiry structures which have developed from sterile flower parts.

Glomérulo maduro de trébol subterráneo (Trifolium subterraneum L.), con dos semillas encerradas en los restos de la inflorescencia, y rodeadas por muchas estructuras rígidas que se han desarrollado a partir de las partes estériles de la flor.

so helping to draw the burr into the ground. In this way subterranean clover buries a proportion of its seed; clearly an adaptation to help germination in a dry environment.

Subterranean clover is self-fertile, and fertilisation occurs before florets open (Morley, 1961). This is considered to have facilitated the retention in the wild of many discreet true-breeding populations, each occupying a landform, edaphic or climatic niche with subtle and unique differences. However Cocks and Phillips (1979) found considerable numbers of divergent strains present in South Australia and consider that this has been brought about by limited cross fertilisation between cultivars of subterranean clover, and possibly also by mutation, since arrival of the species in Australia. In a subsequent study, Cocks (1992) found 67% of a population originally sown in 1938 had diverged genetically, and he considered hybridisation to have been the main cause of this even though this species has previously been considered autogamous

(Morley, 1961).

Gladstones (1966) describes 89 ecotypes collected in the wild from Western Australia. These mainly true breeding populations differ visually in leaflet shape, presence of anthocyanin pigmentation patterns , or chlorophyll free marks on the lamina, presence or absence and prolificacy of hairs on stem, peduncle and pedicel; colour of florets, and presence and number of coloured rings on the calyx. There are also differences in the timing of, and duration of flowering, and the degree of hardness of the seeds.

Suckling *et al.* (1983) and Macfarlane and Sheath (1984) have found that genetic shift has occurred in New Zealand populations since naturalisation, with hybridisation producing a shift in the flowering time of clovers to an intermedíate position between the varieties 'Mount Barker' and 'Tallarook'.

DISTRIBUTION

Subterranean clover occurs naturally as a component of herbaceous cover in áreas of the oíd world having a Mediterranean climate (Katznelson, 1974; *in Rossiter,* 1978). This is a regime with modérate rainfall of 250 to 600 mm per annum falling mostly in late autumn, winter and spring; cool day mean temperatures of 7 to 15 degrees Celsius, followed by a hot dry summer and autumn with day mean temperatures ranging from 16 to 24 deg C. Soils are below wilting point from late spring-early summer until late autumn, a period ranging from four and a half, to six and a half months. Modérate frosts of up to -10 deg C are experienced. The main natural áreas of subterranean clover are in Spain , Portugal, Italy, Greece, Turkey; in the Baltic around the Caspian Sea; Israel, Syria, Ethiopia, Afganistán and neighbouring Far Eastern Countries; in the islands of the Mediterranean; and in North África.

It is also found in áreas with a marginal Mediterranean climate, ie with a temperature regime as described above but somewhat cooler, and where soils are shallow or sandy, with a limited moisture holding capacity, or steep so that rainfall received runs off rather than being absorbed, so that they fall below wilting point for much of the summer. Thus subterranean clover occurs in sandy heathlands in southern England, southern France and similar country bordering on áreas with a true Mediterranean climate.

Coincident with the utilisation of lands in the southern hemisphere as whaling bases and later for settlement from the 18th century onwards, subterranean clover became widespread in áreas of Australia with a Mediterranean climate (Gladstones, 1966). It was first reponed in Australia in 1896 (Davies,1952). and in New Zealand the species was recognised growing in the northern región on steep hill country which dried out in summer (Saxby, 1956). In these instances it is thought that spread was facilitated by the occurrence of subterranean clover in the hay used as fodder for animáis during passage to the new colonies. A proportion of legume seed can pass undigested through the ruminant digestive system- for white clover this is about 8% (Suckling, 1952), and dung disposal at the port of entry would therefore provide a colonising source. In addition the fruit of this clover, the hooked burr, can hang up in the fleece of sheep for weeks until the seed it contains is shaken or rubbed out miles from the point of attatchment.

Since naturalisation, subterranean clover has made a huge impact on agriculture in Australia, where by 1970 there was some 20M ha sown to subterranean clover (Donald, 1970). In New Zealand adoption and use of this plant was slow until the 1930s (Saxby, 1956), but even then and to the present day there is little if any intensive use of subterranean clover as a puré sward, although it is widespread on dry hill country (Suckling *et al,* 1983). Since subterranean clover is an annual, survival, and henee distribution is governed by factors which affect the ability to produce seed. These are dealt with in detail later in this review.

Commercial plantings are in use in parts of the western USA, as well as in températe South África, and several South American countries including Chile, Uruguay and Argentina.

ADAPTATION TO SOIL CONDITIONS

Species adaptation

The natural distribution of the three main species of subterranean clover is to a large degree determined by edaphic factors. T. *sub.* subsp. *sub.* var. *subterraneum* and T. *sub.* var. *yanninicum* prefer acid to neutral soils, while T. *sub.* subsp. *brachy.* var *brachycalycinum* tolerates higher pH, even alkaline soils (Katznelson, 1974; quoted by Rossiter, 1978). Preferred soil conditions for these subspecies are: well-drained and alluvial soils for var. *subterraneum;* stony or ruderal soils for var. *brachycalycinum* and poorly drained alluvial soils for var. *yanninicum* (Katznelson, 1970). Var. *brachycalycinum* is also found on heavier clay soils. The greater tolerance of flooding by var. *yanninicum* (Marshall and Millington, 1967) is a result of several adaptive features of this species including a greater tolerance of root-rotting pathogens (Flett *et al,* 1993), a smaller build-up of ethanol following immersion, and a greater ability of the root system physiology to achieve higher levéis of oxidation when waterlogged (Katznelson, 1970) probably due to the shallow root system.

Acidity, rhizobia and host relationships

Subterranean clover must be nodulated by *Rhizobium trifolü* to thrive. Inoculation of seed with appropriate effective strains of *R. trifolü* is necessary if the soil has not supported *Trifolium* species before (Norris, 1970) or if the resident strain of rhizobia in the soil is not effective. *R. trifolü* can survive in defined solutions from pH 4.0 to 4.6, but grows rapidly over the range 4.7 to pH 5.0 (Loneragon and Dowling, 1958). Nodulation responds to liming or calcium carbonate pelleting of the seed below pH 5.This is largely a requirement for the survival and multiplication of *Rhizobium trifolü* outside the plant but in the rhizosphere prior to, and during initial infection (Hely, 1965). Whilst few soils in New Zealand have an acidity level below pH 5.0 (McLaren and Cameron, 1994a), in southern Australia there is a substantial área of these, amounting to 7.5 M ha in New South Wales alone (Cregan *in* Evans, 1987). Calder (1951) recorded a 10% response to lime in a stony Yellow-brown earth soil with pH 5.5 in southern New Zealand, but this was probably due to the increased availability of phosphate at pH 5.8 after liming (McLaren and Cameron, 1990c).

Twelve liming experiments on acid soils in Australia (Evans, 1987) indícate that the máximum yield of subterranean clover is reached at pH 4.8-5.5. However the responses were variable. This is because acidity affects both host plant growth, and also the process of establishing a functionally adequate nitrogen-fixing symbiosis. In addition soil acidifícation usually results in excesses of not only H ions, but also soluble Al and Mn leading to reduced availability of Ca, Mg and Mo. The growth of the host plant has been shown to be more tolerant of acidity-down to pH 4.5- than the processes of growth in the rhizosphere and infection of the host by *Rhizobia trifolü.* Nodulation was delayed, and nodule numbers reduced by increasing amounts of soluble Al, even at concentrations well below that required to affect root growth (Kim *et al.,* 1985). In this work there was considerable difference in response between the twelve cultivars of subterranean clover involved, which means that there is scope for selection.

Manganese toxicity has been shown to be responsible for a substantial yield reduction in plants well supplied with N but with 45 ppm Mn, as a result of toxicity to the plant and reduced root mass (Evans *et ai,* 1987) although tolerance varied widely between cultivars.

Although most lines nodulate readily with strains of rhizobia available in inoculants, 'Woogenellup' must be inoculated with specific strains of R. trifolü for the most effective symbiosis (Gibson,1968).

Subterranean clover is capable of increasing soil N levéis by as much as 80 kg N ha'. year providing adequate P is available (Watson and Lapins, 1964) and soil acidity is not less than pH 5 (Evans *et al,* 1987). The subsequent yield of crops in a rotation was increased substantially as a result (Heenan *et al,* 1998). Soil N accumulation of 45-65 kg ha' in response to an N fíxation rate of 100 kg ha' was recorded by Helyar *et al.* (1997). Nitrogen fíxation of up to 238 kg ha' was measured by Peoples *et al.* (1998) in a ley rotation, where they obtained large increases in fíxation following winter spraying to remove grasses. The apparent disparity in amounts of N fixed is undoubtedly due to differences in clover yield as a result of climate, soil fertility and management, since both (Dear et al. 1999) and Peoples *et al.* (1998) have found that the amount of N fixed is linearly related to subterranean clover shoot biomass. This was lower when growing with phalaris, but higher in association with lucerne, which had a non-competitive additive effect on N fíxation. Subterranean clover as a puré stand fixed 104 kg N yr^{1}, with lucerne a total of up to 170 kg N yr^{1}, and with phalaris, up to 59 kg N yr' over three years.

Soil Fertility

It is not proposed to cover in detail here this aspect of subterranean clover agronomy. It has been reviewed in depth by Williams and Andrew (1970). However it should be noted that subterranean clover is a typical températe legume in terms of nutrient requirements, and needs adequate levéis of the macronutrients P, S, Mg, Ca, K, Na, and of the micro nutrients Fe, Mn, Cu, Zn, B, Mo, Cl and Si, together with N from rhizobial symbiosis, for máximum productivity (McLaren and Cameron, 1990b). However since seed production is so important for the success of the species, two findings in particular are of importance. Although trace element deficiencies are widely recognised in Australia (Williams and Andrew, 1970), at that date there was little mention of B deficiency. However Dear and Lipsett (1987) have subsequently obtained 5 to 21 fold increases in seed production on acid cropping soils deficient in B, but where foliage analysis did not indicate a shortage. The correction of calcium deficiency on soils with pH 4.2 but where subterranean clover persistence was poor gave an increase of 2-400% in seed yield (Ozanne and Howes, 1974). Developing seeds take up Ca directly from the soil It is probable that the species is more competitive for limited nutrient supplies of P and other anions than white clover or lucerne (Mouat, 1957). In New Zealand Mo deficiency on unlimed virgin soils is widespread (New Zealand Soil Bureau, 1968a), whilst young and overlimed soils can be deficient in B for legume growth (Lobb, 1967).

PHYSIOLOGICAL RESPONSE TO THE ENVIRONMENT

Vegetative response to temperatura.

Growth cabinet studies (Mitchell, 1956a) have shown that subterranean clover has an optimum temperature for growth near 17 deg C, although the response curve is fairly fíat. This is in contrast to that for a températe perennial white clover (24 deg C) and 27 deg C for lúceme (Smith, 1970). However in addition, subterranean clover showed an ability to grow faster than white clover at temperatures below 15 deg C, and down to 7 deg C. This ability to grow actively at moderately low temperatures is typical of races and species of plants from áreas with a Mediterranean climate (Cooper, 1964). It explains the ability of the autumn germinating subterranean clover to utilize cooler autumn-winter conditions for growth, and enables this annual to establish competitive bulk in the limited growth season available to it. At latitude 38 deg S in southern New Zealand, Smetham and Jack (1995) recorded substantial subterranean clover growth rates of 16 -19 kg DM ha' d ^{1} over autumn, 12 kg DM ha' d ' over June and July, and 17-54 kg DM ha' d' from spring onwards. Monthly mean day temperatures for these periods were 9-12 deg C; 5-6 deg C, and 8-10 deg C respectively (Smetham and Jack, 1995). These growth rates were substantially higher than those from lúceme over the cool season in the same experiment, and in the spring- early summer period also, although lack of moisture in the subsoil over the latter period is thought to have severely disadvantaged the lúceme.

At a population of 2000 plants m^2 Silsbury and Hancock (1990) obtained twice as much growth at 20 deg C than at 10 deg C, but no response at higher densities. Similarly, Fukai and Silsbury (1976) found that growth rate was maximised by temperatures between 20-25 deg C although differences were small and occurred only until a closed canopy was reached at LAI 3.0 or 2000 kg DM ha ' Although further increases in herbage mass increased leaf área, it also increased mutual shading, and the photosynthate required for increased respiration progressively absorbed any additional photosynthate produced (Fukai and Silsbury, 1978).

Vegetative response to light

Subterranean clover responds to light in similar fashion to white clover and other prostrate clovers. Artificial shading to one third full daylight caused an increase in petiole length and leaf área together with a reduction of leaf thickness (Mitchell, 1956b). These changes also occured with mutual shading in a puré sward (Smetham and Dear, unpublished). This response allows the species to survive considerable competition in a mixed grass legume sward although the máximum petiole elongation appeared to be

25cm, which can be exceeded by grasses with a long interval between defoliation. In a trae Mediterranean climate most species associated with subterranean clover are limited by the climate to being annuals also. However in quasi-Mediterranean climates with some rainfall occuring in the summer, perennial grass ingress following soil N increase provides severe competition for light, most particularly at the time of establishment following autumn germination (Dear *et al*, 1998). In the summer rainfall áreas of the Australian Tablelands in Queensland, white clover competition has been shown to be a likely cause of lack of persistence of subterranean clover (Smith and Crespo, 1979).

REPRODUCTIVE RESPONSES

Survival and the agricultural success of subterranean clover depends on the production of adequate amounts of seed. The distribution of subterranean clover is therefore determined mainly by features of climate which affect this. Donald (1960, 1970) suggests that in Australia three boundaries can be recognised for subterranean clover. A rainfall boundary, which if too low results in a season of growth too short for the production of sufficient seed. A high temperature boundary, beyond which floral induction and vernalisation fail to occur , and a low temperature boundary which limits the process of flowering. While most subterranean clovers can survive frosts of 7-10 deg C during vegetative growth (Smetham, 1977), at altitude temperatures may be too low to induce flowering (Evans, 1959), and frosts can severely damage flowers and seedsetting (Donald, 1960). In hill country at Canberra, Australia, he considered the upper boundary for subterranean clover to be around 1200 m asi. with seed production being impaired above 600 m. There are indications that the upper boundary is about 900m in the Central Otago área of New Zealand (T.E. Ludecke, pers.com.)

Vernalisation and the timing of flowering

Subterranean clover flowers in response to vernalisation, long days and warm temperatures (Evans, 1959). By mid winter ground-hugging branches (runners) start growing out from the rosette of subterranean clover, and flowers may eventually appear in the axils of leaves at nodes on these runners. Flower initiation in subterrranean clover is dependant on both temperature and daylength. Aitken and Drake (1941) found that the very early flowering variety 'Dwalganup' had initiated floral structures and these appeared at a low node (number four to number seven on a runner) when this cultivar was exposed to a mean temperature of 12 deg C for one to two weeks during the month after sowing and when the photoperiod was 10 hours. Exposure to higher temperatures delayed flowering until nodes 8 to 14 appeared, and above 25 deg C few flowers were formed. The very late flowering cultivar 'Tallarook' however needed exposure to 4 deg C before flowers appeared at a low node; flowering occured at nodes 11 to 18 at higher temperatures, and above 13 deg C no flowers appeared. Exposure to a longer daylength decreased the requirement for cold. Although delayed, 'Dwalganup' managed to flower with initiation temperatures up to 25 deg C and a daylength of 14 hours, but 'Tallarook' remained vegetative above a vemalisation temperature of 18 deg C regardless of daylength (Aitken, 1955a).

In addition to exposure to cold, Aitken (1955a) demonstrated that the requirement for cold was quantitative. Whilst with the early flowering 'Dwalganup', cold exposure was not obligatory, it did flower at a lower node if given 2.5 weeks at 10 deg C, but with no further response beyond that period. 'Tallarook' on the other hand flowered at a high node after 6 weeks at 10 deg C but needed eight to ten weeks at 10 deg C to flower at the lowest node. The pattern of flowering for increasingly lower latitudes predicted from these studies was confirmed by Aitken (1955b) who showed that early varieties delayed flowering, and finally did not flower at all with decrease in latitude and associate higher temperaturer. Mid and late flowering types exhibited this behavior at a higher latitude than the early varieties. Morley and Davern (1956) have demonstrated that cultivars flowering at a time between very early and very late have an intermedíate vemalisation requirement.

From these studies it can be seen that the temperature requirement for initiation constitutes a warm boundary for the species and its ecotypes.This can be determined geographically by reference to climatic data. Donald (1960) quotes field experience which finds that the warm boundary for adequate seed production with the midseason variety 'Mount Barker' coincides closely with the 13 deg C isotherm for July on lowland cióse to the NSW-Queensland border, and the boundary moves further north on the higher altitude, and therefore colder, Tablelands.

Soil moisture and seed production

The spring and early summer months in a Mediterranean environment show a typical pattem of decreasing rainfall and soil moisture, together with increasing temperature and atmospheric dryness. Thus adequate soil moisture for long enough to allow full maturation of sufficient seed is critical to the success of any given variety of subterranean clover. The findings of Aitken (1955a; 1955b) explain how early flowering subterranean clovers flower in late winter-early spring (July-August in the southem hemisphere-SH) (Smetham, 1977), -early enough to produce viable seed in situations where moisture becomes limiting for plant growth six to eight weeks later in spring (September SH) They have little or no requirement for cold to trigger floral initiation. However at the other extreme, late flowering ecotypes flower late because they need some ten weeks of exposure to temperatures below 10 deg C before initiation occurs. These commence flowering in late spring-early summer (mid-October in SH) and need adequate moisture into summer (December SH) for a good seed set. There is a continuum of ecotypes which lie between the extremes of flowering time, having a progressively increasing requirement for cold. The variety 'Clare' has an atypically greater requirement for cold than other midseason flowering types (Morley and Davern, 1956).

In Australia the áreas where moisture becomes limiting early are generally those with a low annual rainfall, and those with late moisture availability are áreas of high rainfall. Thus early flowering cultivars like 'Dwalganup' and 'Geraldton' has been successful in áreas with rainfall as low as 280-300 mm.year', whereas the late flowering 'Mount Barker' is used in áreas with 800-1500 mm.year^{1} (Rossiter and Ozanne, 1970; Quinliven and Francis, 1976). The inland or arid boundary for these cultivars is determined by the length of time during which moisture conditions satisfactory for growth and seed maturation are maintained at any location (Trumble, 1957) combined with local experience of the actual growth period required . For a mid season flowering variety this was estimated to be 7.5 months and for an early type like 'Dwalganup' 6 months (Donald,1970). On this basis for instance the boundary for 'Mt Barker' is not far inland from the southwestern tip of Western Australia, whereas for 'Dwalganup' the boundary is some 380 km inland and runs from Hopetown to near Geraldton.

Since germination of subterranean clovers occurs with the advent of autumn rains, all varieties commence growing at much the same time regardless of whether they are early or late flowering. As the latter have to grow for a longer period of time in order to acquire the cold requirement for vernalisation, it follows that they grow vegetatively for longer before flowering and henee tend to have a higher vegetative yield than early flowering ecotypes.Thus Aitken (1955a) found that early ecotypes produced a first flower in the axil of the third fourth or fifth leaf on any stem, whereas late varieties had produced 12 to 15 leaves before flowers appeared. In addition, and for the same reason, the later flowering varieties develop more inflorescences (Donald and Neal Smith, 1937) and henee have a greater potential for seed production. This is only realised however if the moisture environment allows this to happen (Rossiter, 1959). In addition to an ability to flower and produce adequate seed before dryness sets in, strains differ in the rate and pattern of production of flowers. Rate of inflorescence production was found by Francis and Gladstones (1974) to be the major determinant of seed production in a study of 24 strains, ranging as it did from 110-240 day ' m^2 inflorescences produced.

Máximum seed production, and henee success in a given área, will be given by a variety which just manages to mature adequate seed , rather than an earlier flowenng type, especially since excess moisture during and after seed maturation can lead to large losses following imbibition and rotting of the seed (Collins *et al,* 1966; Archer, 1990).

With progression from spring to summer, flowering and seed maturation continué until plants die from lack of moisture.

Control of germination

There appear to be several mechanisms which opérate to prevent the germination of subterranean clover seed until conditions are likely to be suitable for good survival of seedlings. The first group of these promote embryo dormaney, while the second; the development of hard seed, physically prevenís germination until the autumn, or in some seed until autumn one or more years later.

Embryo dormaney

Embryo dormaney is promoted by high temperature, inhibitory substances in the seed coat, and by an absence of high concentrations of carbón dioxide; all of which have been shown to prevent the germination of fully imbibed subterranean clover seeds. Unlike hardseededness, embryo dormaney is a behavior which only proteets seeds immediately after maturity. It is a mechanism which is of advantage to a plant growing in a summer-dry environment, particularly in áreas where there is sometimes likely to be enough summer rain to set off germination, but not enough to sustain the seedlings.

At temperatures above 20 deg C fully imbibed seeds of subterranean clover fail to germinate, although they do so readily at 10 deg C (Loftus Hills, 1944). Strains vary in their response, which is under genetic control. Some eg. 'Geraldton' and 'Dinninup' have greater dormaney than others eg. 'Woogenellup' and 'Mt Barker' (Taylor, 1970). Protection against widespread loss of seedlings is afforded in summer, but by autumn (late March-April in the southern hemisphere) day temperatures have declined enough to allow germination of soft seeds at a time when survival is almost guaranteed to oceur.

Germination in the autumn has al so been shown to be promoted by exposure to high levéis of $C0_2$ likely to be experienced in the soil , but not on the soil surface, and to leaching with water (Taylor, 1970). In February, continuous leaching with running water, or confinement in a sealed cotainer with water, enabled substantial, though with varietal differences, germination even at 30 deg C, whereas merely soaking in water gave only 8% germination. At 20 deg C leaching resulted in complete germination. This requirement for leaching, equivalent to about 1 Omm of rain, before full removal of the inhibitor occurs ensures at least some continuity of soil moisture to help seedling survival. Walker (1971) has some evidence that the purple anthocyanin in the seedcoat may act as an inhibitor of germination, and it could be assumed that this would be removed by any leaching process.

Development ofthe hardseeded condition

Subterranean clover has the ability, like most legumes, to produce seed which remains unable to absorb water and thus germinate, for a period of weeks to years after maturation. A comprehensive review of seed coat impermeability was made by Quinliven in 1971. The facility to produce hard seed is a mechanism which allows the species to survive seasons where seedlings are lost due to drought or pest attack or where seed production has failed. It particularly protects against premature germination following summer rains in less than true Mediterranean climates. Softening of a proportion of this hard seed in the next season then allows the species to re-establish. For instance (Donald, 1959) found that 6% of 'Dwalganup' seed survived and germinated in the next season after formation; while *2%* still germinated after four years in the ground.

Hard seed is a condition obtained when the moisture content of the seed has dropped to a critical low level. As the seed matures and dries the testa shrinks and becomes impermeable at a moisture content of around 14% of dry weight. Thereafter structures deep within the hilum, or scar of attachment of the seed to its stalk (Bell, 1991), and surrounding the longitudinal groove seen in the pit of the hilum (Figure 3), act as an

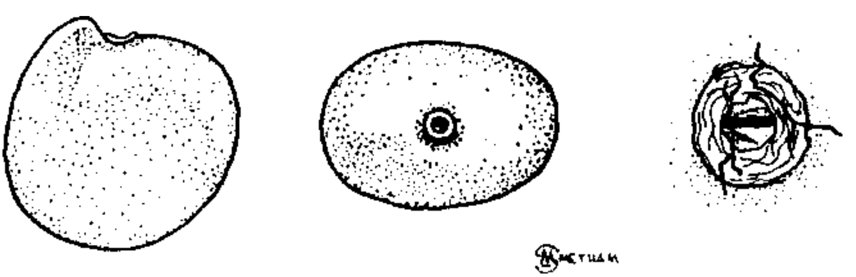

FIGURE 3

The seed of subterranean clover *(Trifolium subterraneum* L. subsp. *subterraneum* van *subterraneum)* in elevation (left), and plan (middle), looking directly down at the small disc which is the hilum. The enlarged hilum (right) shows in more detail its fibrous nature; the deep pit in the centre, and the slit-like aperture in this.

Semilla de trébol subterráneo (Trifolium subterraneum L. subsp. subterraneum var. subterraneum): Vista lateral (izquierda), vista del hilo (en medio), y vista de detalle del hilo (a la derecha), en la que se muestra su naturaleza fibrosa, el profundo hundimiento en el centro y el orificio de apertura, de forma alargada.

hygroscopic valve, preventing water from entering but allowing moisture out. The moisture content of the seed eventually reaches equilibrium with the driest conditions to which it is exposed (Hyde, 1954). Seeds need to reach a low level of moisture to attain an irreversible impermeability (Quinliven, 1971) which for subterranean clover appears to be 5-7%.

Hardseededness is maximised when the seed matures slowly over a long growing season and in the absence of moisture stress (Aitken,1939; Quinliven, 1965). Seeds matured under these conditions subsequently soften more slowly. An additional mechanism contributing to impermeability is the presence of a continuous layer of suberin, laid down over the coloured part of the seed coat (Aitken, 1939) which further prevenís ingress of moisture into the seed. However if moisture stress occurs during seed maturation, then this suberin layer may be thinner and even discontinuous. It is evident therefore that if soil moisture déficit occurs soon after flowering has started, subsequent seed may lack the protection of hardseededness. Sporadic midsummer rainfall may promote germination of this seed only to be followed by death of the seedlings in subsequent drought. Studies over a twelve year period by Taylor *et al.* (1984) of seed losses from drought-so called "false strike", showed that these averaged 24% of all seed set.

Provided adequate soil moisture to mature seed is available over the main flowering period, at the end of this time hardseededness will be at a máximum. However if excessive moisture is present during seed maturation and subsequently, hardness of seed fails to develop, and considerable amounts of seed can be lost to fungal rots (Collins *et al*, 1966; Archer, 1990).

Máximum levéis of hardseededness occur in late spring early summer, with a significant difference between strains and between maturity groups, although all groups exhibit a relatively high percentage of hardseed at this time. In the Mediterranean climate of Western Australia Quinliven and Millington (1962) measured hardseededness in the first month after cessation of growth in mid November of 95-97% for early varieties through to 59% for late flowering cultivars (Table 1). That early-flowering ecotypes should exhibit a higher level of hardseededness than later flowering ones (Gladstones,1967) seems anomalous since the chances of summer rainfall in environments suited to early varieties are slight. However this environment allows the full development of hardseededness, whereas in many situations the high relative humidity at seed maturation for late varieties prevenís much of this seed from drying down to the critical level for hardseededness to become complete.

TABLE 1

The proportion of hard seed possessed by nine cultivars of subterranean clover at seed maturity; after four months exposure to summer temperatures in Western Australia, and after six months of exposure in an oven, to a diurnal fluctuating temperature regime of 15 deg C night/60 deg C day temperatures (Quinliven and Millington, 1962).

Proporción de semillas duras de nueve variedades de trébol subterráneo en el momento de la maduración de la semilla, después de estar cuatro meses expuestas a las temperaturas de verano del Oeste de Australia, y después de una exposición de seis meses en una estufa a una fluctuación de temperaturas noche/día de ¡5/60 "C.

Cultivar	Early summer (% hard)	Four months in field (% hard)	Six months in oven (% hard)
Geraldton	97.0	64.7	29.3
Dwalganup	96.5	**62.4**	10.3
Burnerang	82.4	**52.4**	28.8
Carnamah	95.5	**51.2**	8.5
Morocco	80.7	**35.3**	26.4
Woogenellup	86.0	**34.4**	2.8
Palestine	89.2	**29.6**	3.1
Bacchus March	71.0	**16.5**	0.2
Mount Barker	58.8	**11.1**	2.1

Decline of hardseededness

With exposure to sufficient variation between day and night temperatures, hardseededness declines steadily in any seedlot between maturation and the following autumn so that the majority of seed can germinate at a time of year when this will be followed by adequate moisture to ensure survival of the seedlings. Softening to allow germination is influenced positively by the amplitude of the diurnal temperature variation and the magnitude of the high temperatures reached. Quinliven (1961, 1966) found that. softening was significantly faster under a 60 deg C day and a 15 deg C night regime than a 46 deg C /l5 deg C diurnal variation. There was however no increase in rate of softening when the day temperature was 73 deg C. The regime causing the most rapid softening approximates soil surface temperatures in many parts of southern Australia in mid-summer eg. Griffith, New South Wales with a mean January (SH) day máximum temperature at lpm of 51 deg C and a 3am mean mínimum night temperature of 25 deg C (Quinliven, 1961). Likewise Taylor (1984) recorded a daily mean máximum in January of 58 deg C; and a minimum of 13 deg C in wheat cropping country in Western Australia. Exposure to cooler conditions during the summer will clearly slow up the softening process and Evans and Hall (1995) found that 12-31% of the original seed was still hard after six years exposure to the cooler conditions of Tasmania with summer monthly máximums of 20-23 deg C and mínimums of 10-11 deg C. The rate of softening was the same for all varieties tested, but was twice as fast at low, than high altitude.

Hagon (1971) found that the diurnal variation of temperature responsible for softening caused altérnate expansión and contraction of the tissues comprising the strophiole, resulting in the mechanical rupture and chemical degradation of this región of the testa (Young, 1985), allowing moisture to enter the seed. Unfortunately Hagon did not describe the location of the strophiole, although he did establish that this was the only point of entry for moisture after the softening process. Bell (1991) describes the strophiole as a conspicuous swollen fleshy addition on that part of the ovule stalk fused with the outer integument, and known as the raphe. Diagrams in Córner (1951) and Hagon and Ballard (1970) where the alternative ñame "lens" is used, indicate that this structure is in line with the tip of the radicle and the hilum, but on the other side of the latter, and is a point of weakness in the testa. Only at this point did impaction soften hard seeds of *T. Subterraneum* L. (Hagon and Ballard, 1970) and *Melilotus alba* L. (Hamly, 1932) by splitting palisade cells in the testa, allowing water to enter.

Signifícant variation occurs between strains for the rate and pattern of decline of hardness, and Quinliven and Millington (1962) record that while the initial levéis of hardseed in the early flowering 'Geraldton' and late flowering 'Mount Barker' were 97% and 59% respectively, after four months in the field these had dropped to 67% and 11% respectively, with similar behavior over the range of intermedíate varieties (Table 1). So-called "soft" varieties of subterranean clover are generally those which exhibit a faster decline of hardseededness, and consequently have a relatively low proportion of hard seed by autumn.

Hot summers with a wide diurnal amplitude of temperature were noticed by Quinliven (1965) to cause more rapid softening in the autumn, and in 1981, Taylor proposed that softening is a two stage process, being influenced initially by the valué and duration of constant temperatures experienced in the summer of seed maturation. All temperatures from 15 deg C to 80 deg C accelerated softening in the following autumn, but 60 deg C and above had most effect, with seed softening in the autumn after only one to three cycles of alternating 60/15 deg C temperatures in the second stage of the process. Taylor (1981) suggests that the initial steady temperatures cause the thermal degradation of the strophiolar región, while the fluctuating temperatures of the second stage physically disrupts the strophiole allowing the entry of moisture. Considerable variation exists in the rate of autumn softening; enough to allow selection for this trait to be benefícial (Smith *et al,* 1996).

Burial in soil reduces the amplitude and extremes of temperature experienced by the seed and so also slows the softening process. Whilst the soil surface diurnal variation of temperature was 58/13 deg C, at 2cm depth it was reduced to 50/17 deg C and at lOcm 35/24 (Taylor, 1984). 95% of 'Geraldton' seed, initially hard, germinated during the fírst year on the surface, but only 25% had softened during four years buried at lOcm (Taylor and Ewing, 1988). There are varietal differences, and in the same investigation 'Nungarin', also an early variety, still had 20% of hardseed ungerminated after three years on the surface. Taylor (1984) found that few hard seeds of 'Yarloop' survived more than three years at any depth.

Conditions of storage and burial also affect the rate of hardseed breakdown and Taylor (1984) found that softening was accelerated by up to four times after burial for very long rather than short periods in soil, and he postulated a pre-conditioning effect of storage on rate of softening. He found that the rapid within year autumn softening of annual medies only oceurred after 16 weeks of exposure to 60/15 deg C daily cyeles of temperature. However in view of his later findings (Taylor, 1996) it is more likely that the temperatures during the season of formation were responsible for this result. This accords with his 1981 findings with subterranean clover which caused him to propose a two stage model of softening.

Differences in rate of decline also oceur because of different amounts of herbage topcover. This progressively reduces the magnitude of the temperature fluctuations experienced at the soil surface and consequently reduces the rate of softening. For example máximum temperatures measured in January by Quinliven and Millington (1962) averaged 55.6 deg C under very light top cover, but was only 43 deg C under heavy topgrowth. This would reduce the softening rate by some 30% in a variety like 'Mt Barker' (Quinliven, 1961). Grazing to reduce herbage cover can clearly be used as a management tool to accelerate softening should this be necessary as it could be in áreas with a cooler summer.

Burr burial

The ability of subterranean clover to bury seed in a burr below ground level results in the seed being in a better environment for maturation and germination. Hardseededness is lost more slowly and the seed is to some extent protected from grazing animáis, although sheep quickly learn to scrape away soil in order to get at the burrs in time of feed shortage.

Burial results in heavier and more viable seeds, and in more seeds per burr giving a higher seed yield partieularly in early flowering varieties (Yates, 1958). The burial of burrs, which occurs as the seed is maturing, protects the developing seed from the adverse effects of high temperature and low humidity and provides a more favourable moisture regime (Yates, 1957). Burial in the field resulted in a fíve fold increase in seeds per burr, and a variable but always positive increase in the weight of seed produced. In addition it tends to aid the development of hardseededness (Aitken, 1939). When burial was artifícially prevented, the number of burrs produced, and the weight per seed was reduced in all three subspecies of subterranean clover, leading to a drastic fall in weight of seed produced, often to less than 50% of the weight obtained when natural burial was allowed (Bolland and Collins, 1986). Defoliation significantly increases burial (Walton, 1975) and Collins *et al.,* (1983) found burial increased with defoliation during flowering to include 95% of all seed formed.

There are genetic differences in the ability to bury burrs, with early types burying a high proportion and late types a low proportion of burrs (Francis *et al,* 1972). Burial is to some extent dependant on soil surface roughness and soil texture and is facilitated by the presence of cracks and clods, and is greater in sands than in clays. The species *T sub.* subsp. *sub.* var. *subterraneum* has a greater ability to bury burrs than *T sub.* var. *yanninicum,* which in turn is better than *T sub.* subsp. *brachy.* var. *brachycalycinum* (Francis *et al,* 1972), which only manages to bury burrs in cracks formed in cracking clays. However this may be a function of the edaphic adaptation of each subspecies: var. *subterraneum* to sandy light soils; var. *yanninicum* to rather wet soils subject to flooding, and var. *brachycalycinum* to stony ruderal soils , and the relative ease or difficulty of burial in these respectively.

Burial also aids survival of seed from year to year (Taylor and Ewing, 1988). For instance only 5% of hard seeds of 'Geraldton' survived after one year on the soil surface but 75% were still present four years after formation, when buried at lOcm. This is because softening is slowed down since radiant heat receipt is reduced by the insulating effect of both soil and trash.

THE USE AND MANAGEMENT OF SUBTERRANEAN CLOVER IN AGRICULTURE

History and manner of use

Subterranean clover was regarded as a weed in New Zealand until around 1925, but from that time on increasing experimentation was made with subterranean clover in áreas of the country where soil moisture severely limits plant growth for from one to four months over summer (Saxby, 1956). This may be caused by low rainfall, shallow soils, and high summer temperatures of 26-32 deg C or a combination of any or all of these. This environment makes subterranean clover a well-adapted choice as a persistent legume base for pasture. Suitable áreas have a mild winter with mean day temperatures of 5-10 deg C, with some frosts of up to 10 deg C, which allows cool-season growth without causing the death of plants, although these winter day temperatures are not as warm as those experienced in a true Mediterranean climate (Gentilli,1971), and neither are the summer temperatures of 16-19 deg C as high as those experienced in a Mediterranean climate. The difference between summer day and night temperatures is likewise lower at only 8-10 deg C.

Following encouragement from Professor E. R. Hudson of Lincoln College, Canterbury, New Zealand, and research at the same institution (Calder, 1951), subterranean clover use from the early 1930's was extended to the Canterbury Plains and other fíat to rolling áreas of shallow, stony or sandy soils receiving 600 mm to 750 mm annual rainfall (RF), some of which falls in summer, and drying below wilting point for most of the time between November and March-April (Saxby, 1956). This resulted in the development of a particularly successful farming system, involving much earlier lambing, starting in July, so that a proportion of lambs could reach killable weight; fat off their mothers, before the subterranean clover-based swards died off in early summer-late November in the southern hemisphere. Previously, short-lived annual types of white clover were the legume base for much of this country, which supported a largely annual grass population. The use of subterranean clover as the dominant pasture species, increased carrying capacity more than threefold to nearly six ewe equivalents ha' (Calder, 1951).

From the late 1940's subterranean clover was increasingly sown on hill country after bush clearance and burning, being broadcast by hand initially (Saxby, 1956), but later using aircraft. Its use on dry sunny-face hill country of the North Island was encouraged by early grassland experts like Sir Bruce Levy (Levy, 1970) and the species survives on such country today. Although the annual rainfall on most of this land is 1000-2000 mm yr', effectiveness is markedly reduced by wind; the high summer temperatures, and the steepness of the terrain, leading to summer drought, more particularly on country with a sunny aspect.

Although in 1936, Levy and Gorman recommended that more productive cultivars be used, the late season flowering 'Tallarook' and the mid-season flowering 'Mount Barker' were used but only because seed of these varieties was readily available. These two varieties still constitute the majority of stands in New Zealand (Suckling *et ai*, 1983; Macfarlane and Sheath, 1984). In New Zealand use of subterranean clover has been exclusively in a mixed stand, generálly with a perennial ryegrass.

In Australia subterranean clovers arrived with the fírst whalers and settlers and their sheep early in the 19th century (Gladstones, 1966). Plants spread into áreas with low to modérate annual rainfall of 280mm to 1400mm, and a Mediterranean climate; mild winters with a mean day temperature of 7-15 deg C; hot summers with mean day temperatures of 18-24 deg C, and relatively little rain for the four to six months of summer (Gentilli, 1971b). Different varieties have been recognised since 1907, when 'Mount Barker' was first commercialised (Symon, 1961) with most cultivars being named after districts or properties in Australia where they were first identified (Donald, 1960). Cocks and Phillips (1979) list 18 cultivars in commerce by 1976, 15 of which were strains naturalised since settlement; two were interstrain hybrids, and one was an overseas selection. These included the early flowering 'Dwalganup'; commercialised in 1929; and 'Yarloop' (1947), midseason-flowering 'Bacchus Marsh' (1940) and 'Nangeela' (1961), and mid to late-season flowering 'Mount Barker' (1907) and 'Tallarook' (1935).

In Western Australia only 10-30% of rainfall occurs in summer (Gentilli, 1971b), and apart from the extreme tip of the land área, is too dry and hot in summer for perennial grasses to survive. Annual grasses are sometimes sown with the subterranean clover, but pastures on acid soils are mainly sown to puré subterranean clover (Rossiter and Ozanne, 1970) to build fertility for wheat, and for grazing. Between the 450mm rainfall isohyet and the inland arid boundary for subterranean clover which is around 280mm, early varieties are used. Between 450 mm and 750 mm annual rainfall early mid season varieties are used, while late types are sown in the extreme western tip where rainfall is greater than 750 mm (Gillespie and Nicholas, 1991).

In eastern Australia summer rainfall is a significant proportion of the total, increasing from 30% of total in the western plains to around 40% on the slopes and 60 % on the tablelands of the main divide (Gentilli, 1971a). Perennial grasses such as phalaris are used in mixture with subterranean clovers where annual rainfall exceeds 560mm, while perennial ryegrass survives above 750mm and in irrigated pastures with subterranean clover on the tablelands and in parts of Tasmania (Moore, 1970). Subterranean clovers are sown puré on acid soils in áreas of eastern and southern Australia with rainfall of less than 550mm, down to around 350mm, mostly as a fertility building break in a cropping regime but also to feed sheep and cattle. Such swards are rapidly invaded by annual broadleaved weeds and grass following the N build up associated with legume growth. In both the west and east of Australia, free draining soils which are not too acid, with rainfall above 350 mm and a significant proportion of this occurring in summer, are often sown to a mixture of subterranean clover and lúceme.

Although annual grasses like *Lolium rigidum* Gaud. are sometimes encouraged in mixture with subterranean clover, grass species including annuals are not favoured in the restorative years of a cropping rotation because some, including the weed species in the genus *Vulpia,* are carry-over hosts of *Gaeumannomyces graminis* var. *tritici,* which causes take-all disease in cereal crops (Leys *et al,* 1993). This can severely reduce wheat yield. (B.S. Dear, pers. com). Subterranean clover is used in these áreas as a puré sward down for three to five years in rotation with one to four crops of wheat. Depending on the time of the "finish" ie when plants die having used all available moisture, and the time of the "break" ie. when substantial rain first falls in autumn, the production of these puré swards lies between 4 and 11 t DM ha' (Dear *et al,* 1993). The relatively low numbers of sheep on these farms lamb in the autumn in early April; and graze on wheat stubble to the end of April, or until the subterranean clover on the rest of the property has re-established and is strong enough to be grazed in early June. Grazing of the subterranean clover continúes over spring-early summer while the clover is flowering. although the increase of growth at this time means the grazing pressure on the subterranean clover pastures is not particularly great. Weaned ewes utilise crop residues from wheat harvest in December (mid-summer) through to the end of April. By mid-November the subterranean clover topgrowth has died off, leaving considerable bulk of leaf, stem and some burr, and this is progressively utilised by sheep until subterranean clover germination occurs in early April (B.S. Dear, pers. com.).

Seed production for agricultural success

Cárter and Cochrane (1985) propose that for the continued agricultural success of a stand, a mínimum reserve of 200kg.ha seed should be maintained in the soil. Rossiter (1966) quotes a considerably higher figure of 600 kg ha' but this was to enable a variety to survive in competition with non-leguminous sward components. More recently Dear *et al,* (1993) maintain that seed reserves of early mid-season flowering cultivars in áreas receiving 500-600 mm annual rainfall with rain in summer, should be at least 500 kg ha'.

Seedling densities of at least 1000 plants m^2 (Taylor *et al,* 1991: Silsbury and Fukai, 1977) and up to 5000 plants m^2after full germination in the autumn (Taylor *et al,* 1984) are suggested as being necessary for highest winter and total DM production. If germination is 100%, then with a seed weight of 7 g/ 1000, 70 kg ha^1 of seed will be required to give 1000 seedlings m^2. However in Australia most subterranean clover cultivars still retain a degree of hardseededness at the break in the autumn. This ranges from up to 45% in early cultivars e.g. 'Dalkeith'; 35% in early mid-season types e.g. 'Seaton Park'; and 15% in late varieties like 'Mount Barker' (Collins *et al,* 1984). When allowance is made for this, the minimum amounts of seed required just before germination in the autumn are therefore 127 kg ha', 108. kg ha', and 82 kg ha' for early, mid and late-season flowering cultivars, respectively. Substantial loss of seed reserves can occur when seedlings are killed by drought in a false strike (Rossiter, 1966; Taylor, 1972; Taylor *et al.*, 1984) The latter authors recorded a mean loss of 24% over twelve periods of regeneration. If such losses are assumed to be at least 25%, the mínimum amount of seed required needs to be increased to 168 kg ha ', 144 kg ha', and 109 kg ha'. Up to 83% of seed can be consumed when sheep are grazed on pastures after the herbage has dried off, (de Kooning and Cárter, 1989). If it is assumed that 50% of seed is lost in this way, then the minimum amount of seed which needs to be set becomes 336 kg ha' for early, 288 kg ha^{1} for mid-season, and 218 kg ha^{1} for late-flowering cultivars. Although theoretical, these threshold figures for total seed production can form a useful benchmark when assessing the performance of subterranean clover lines in comparative triáis.

No allowance has been made for seed quality, diseases or pests. Insects like crickets and ants may remove and consume seed. Cambell (1977) has measured the removal by seed-eating ants of between half and two thirds of the seed sown following aerial oversowing on Tablelands tussock country. However as opposed to seed lying on the ground surface, natural burial of seed in burrs may mitigate against predation . In an investigation of seed loss between seed set and the following autumn , false strike at 23% accounted for all seed losses in spite of seed eating ant *{Pheidole megacephala* Fabr.) colonies being present on the experimental área (Smetham and Dear, unpublished data). Since insect predation has not been widely reponed it is therefore not allowed for in the amendment of the amount of seed required for success. It should be noted that the above calculations take no account of hardseed which may carry over from one season to the next.

Seed production is reduced by competition with both grass and legume perennials when these are grown with subterranean clover. Although water in spring was not a factor affecting seed yields, these were strongly correlated with the amount of light reaching the clover component, resulting in a 50-75% reduction of seed yield at the highest density of 35-40 perennial plants m^2 (Dear *et al*, 2000). Even so, seed yields were with one exception all at or above 400 kg ha' and so above the minimum for success proposed above. In addition, although seedling numbers were depressed by competition, they generally exceeded 750 seedlings m^2 counted at the highest perennial density, and were often above 1000 seedlings nr^2, so exceeding the minimum suggested for máximum DM production.

Under the moderately dry (580 mm/yr annual rainfall) conditions at Wagga Wagga, New South Wales, the use of herbicides has been shown to increase stomatal resistance and reduce leaf área (Dear *et ai,* 1996) causing a water-saving effect and resulting in up to twice as much seed being produced. However at wetter sites seed production was either not affected or depressed by up to 66% (Sandral *et al,* 1995).

The effect of grazing on seed production

Over the last 20 years in Australia there has been a growing awareness that many subterranean clover pastures are not as productive as they once were. Among the several factors blamed for the decline of this legume has been the increase of stock pressure on pastures, particularly those grown as part of a cropping rotation (Cárter *et al,* 1982). Sheep have been shown to progressively deplete by ingestión seed reserves of subterranean clover during the grazing of dried-off herbage in summer and autumn (de Koning and Cárter, 1989), but grazing during flowering and seed maturation has a major impact also. Several workers (Collins, 1978; Collins, 1981; Collins *et al,* 1983; Rossiter, 1972) have recorded an increase in seed yield in response to defoliation, or defoliation and grazing (Rossiter, 1961) or grazing alone (Rossiter and Park, 1972; Bolland, 1987) up to first flower appearance. However grazing (Rossiter, 1961) or defoliation (Collins, 1978; Collins *et al,* 1983) during flowering decreased seed yield. Increases in yield with defoliation before flowering were not however obtained with spaced plants (Rossiter, 1972) or sparse swards (Walton, 1975) because subsequent flower numbers were reduced, a result the reverse of that obtained with dense swards (Collins, 1978). Insufficient light penetrating the canopy of dense swards to allow the full potential for seed production to be expressed is the probable explanation since Collins *et al.* (1978) obtained a large reduction in seed yield correlating with a reduction in inflorescence numbers where swards were shaded during flowering.

Rossiter (1972) counted 17% more flowers as a result of defoliation during the early part of the flowering period , but more flowers still, and 27% more seed where grazing and defoliation were stopped before flowering commenced. Subterranean clover seems to respond in similar fashion to white clover, the stolons of which branch to give more sites for inflorescence initiation (Beinhart, 1962) and more flowers at axilliary sites (Zaleski, 1964) when light intensity at stolon, ie ground level, is high. This hypothesis tends to be confirmed by Hagon (1973) who obtained more seed from defoliation, but only where the leaf área index of the sward exceeded that for full light interception.

However there is little information on the effect of grazing throughout the whole period of flowering. Conlan et al., (1994) obtained a reduction of seed yield with some varieties, particularly 'Clare', but in others there was no effect. Grazing was intermittent and in the most severe treatments still left an LAI of 2.7-3.6 which equates to full light interception (Fukai and Silsbury, 1976) and is unlikely to have unduly stressed plants.

Reductions of 30-50% in seed yield were obtained by Young *et al,* (1994) when grazing was continued through to pod formation, but here again grazing was periodic and at long intervals. The most significant fínding of this study was that spring DM yields accounted for 90% of the variation in subsequent seed yields.

One of the few investigations where grazing was continuous during the flowering period (Smetham and Dear, unpublished) demonstrated a large reduction in seed following continuous severe grazing. Stocking so that 1405 kg DM ha' of herbage mass was left after grazing (equivalent to a Leaf Área Index (LAI) of 0.5, or roughly half a layer of leaf per unit ground área) ie very severe defoliation, allowed only 71 kg ha' seed to be produced. Maintaining a herbage mass of 1613 kg DM ha' (or an LAI of 1.5, equivalent to leaving behind roughly one and one half layers of leaf per unit ground área), resulted in significantly more seed being produced (324 kg ha'), while no grazing gave a seed yield of 1254 kg ha^1. More florets were produced when the sward was grazed, but at the severe level of defoliation relatively more flowers were consumed by grazing than at the intermedíate level. This study showed that there were differences between varieties in the height at which the inflorescences were presented, and henee susceptibility to removal by grazing. Nevertheless, the study showed that moderately severe continuous grazing can be practised during flowering, and still allow sufficient seed to be produced for the stand to be agriculturally successful. In addition some grazing, both before and during flowering, is to be preferred to none if seed production is to be maximised.

The impact of hardseededness on performance

The high summer temperatures and considerable diurnal amplitude of temperature variation of a Mediterranean climate causes the hardseededness developed during seed maturation to decline to much lower levéis by autumn (Quinliven and Millington, 1962; Collins 1984, and Table 1.), leading to substantial germination at this time. Such conditions exist in Western Australia with February mean daily temperatures of 18-27 deg C and a diurnal range of up to 17 deg C (Rossiter and Ozanne, 1970), and mean extremes up to 48 deg C (Gentilli, 1971b). Summer temperatures are much the same in South Australia, and south-eastern Australia west of the main divide on the slopes and plains. However on the eastern Tablelands, and in much of Tasmania, temperatures are not as high, with a mean daily máximum around 20 deg C; and a diurnal variation of about 13 deg C (Gentilli, 1971b). As a result the rate of hard seed breakdown is reduced, so that in the autumn hardseededness is still quite high and populations of seedlings are very much below optimum (Fitzgerald, 1987; Evans and Hall, 1995). This is further compromised by the grass often grown as a companion species with subterranean clover in these áreas, due to the extra insulating effect of this additional herbage cover.

The problem is even more acute in New Zealand with mean daily máximum temperatures of 16-19 deg C and a diurnal range of 9-10 deg C although Central Otago, possessing a more continental climate has an increased range of nearly 13 deg C (Gentilli, 1971b). Smetham and Wu Ying (1991) consider this to be a major impediment to the success of subterranean clover in New Zealand, although the protection against loss of seedlings frequently recorded in summer (Sheath and Macfarlane, 1990a) afforded by hard seed is still very much required. In the major study of subterranean clover cultivar performance on North Island hill country (Williams *et al,* 1990) a typical result was a population of 107 seedlings m^2 in March from 1540 seeds m^2 counted in January (Sheath *et al,* 1990). Unfortunately no measurements of hard seed were made, so that the losses from false strike cannot be seperated from the lack of germination due to hardseededness, although in another study 30-50% of seedlings appeared to die from drought (Sheath and Macfarlane, 1990b).

Smetham (1980) has recorded hardseededness in the autumn of 76% in early varieties and 62-72% in midseason varieties from dry hill country, and calculations using the figures of Hoglund (1990) for cultivars grown in the same área gave much the same range of hardseededness (Smetham and Wu Ying, 1991). The área experienced monthly mean máximums of 20-25 deg C in mid to late summer, with minimums of 9-13 deg C. These temperatures are well below those promoting rapid loss of hardseededness (Quinliven and Millington, 1962) and resulted in low populations of 285 seedlings m^2 from 403 seeds (31% hard), to only 120 seedlings from 1494 seeds (92% hard) (Smetham and Wu Ying, 1991). Even the most hardseeded varieties, subjected to a range of day /night temperatures in growth cabinets gave up to 57% germination after only one month of 50day/ 5 night deg C temperatures (Smetham and Jack, unpublished), thus reinforcing the hypothesis of a slow rate of breakdown due to low temperatures and a small diurnal range.

An evaluation of 47 lines in a dry South Island, New Zealand environment (Smetham *et al,* 1993) gave a similarly high level of hardseed. In mid-autumn twenty-two lines were 75-100 % hard, while 37 had hardseededness above 50%. However whilst these levéis were high, the amounts of seed formed by some lines were also exceptionally high. Consequently 14 lines germinated to give more than 1000 seedlings m^2 and could therefore be judged to be successful in the agricultural sense. Selection of lines giving high enough yields of seed so that adequate numbers of seedlings are obtained, in spite of a high proportion of hardseededness, is one solution to the problem of overly hard seed in subterranean clover grown in cooler districts.

Hardseed carryover

A commonly held view is that hardseededness ensures that there will always be seed for next year if the majority of the current seed set is lost. However whilst this may ensure the biological survival of a given ecotype of subterranean clover, it does not guarantee agricultural success, when this is defined as the production of at least 1000 seedlings m^2 in autumn.

Firstly only small percentages of seed soften to germinate in later years, particularly if they are buried, since this slows the rate of softening, although this does vary with variety. Taylor (1984) found that most hard seeds of 'Yarloop' germinated within 3 years at any depth of burial, while few seeds of 'Northam' did so. Whereas 92% of the seed set by four subterranean clover varieties germinated in the fírst autumn, only 6% (or 12 kg ha' seed) did so in the second autumn, giving only 193 seedlings m^2 (Donald,1959), quite insufficient for agricultural success, but more than enough for biological survival.

Secondly, there is some evidence (Chapman and Anderson, 1987) that the viability of hard seed does not remain high for long. Using white clover, they found that of seed which was originally 75% hard, only 25% remained seven months after burial. 25 % was recovered and 22% had germinated, but 53% was lost to unknown causes. A half life of 1 year was postulated, and few seeds were found to have survived for more than two years. Taylor (1984) found some evidence for the microbial decomposition of hard seed buried in soil, with a mean loss of 3%, although in one batch, 49% of seeds died after one year buried at 6 cm. Considerable loss of seed was also recorded by Rossiter (1966), who was only able to account for 35-44% of the seed sown, as having germinated or was still hard after 5 years in the field. This constitutes considerable mortality.

On purely theoretical grounds the environment to which an embryo is subjected inside a hard seed is not one which is likely to ensure a high rate of survival . The seedcoat has limited permeability for oxygen; no permeability for water, and may, if on the surface of the ground, be subjected to near, or above temperatures normally lethal for any plants (Levitt, 1956). At Griffith, New South Wales the bare soil surface mean máximum temperature at 1 pm for one week's observations in mid January was 51.2 deg C (Quinliven, 1961), while mean weekly máximum temperatures at the soil surface were 55-59 deg C during February and March for a site near Perth, Australia (Smith *et al,* 1996). With increasing depth of burial however survival of hard seed is progressively greater, and whilst Taylor (1984) found that on the surface little hardseed remained hard for long, when some varieties were buried at 10 cm more than 70% of the hard seed survived for three years or more.

The apparent losses of seed from a hill site in Canterbury, New Zealand tend to support the hypothesis that hard seed may not survive as well as has been supposed. For instance less than 50% of seeds were present in February 1985 when compared to the 1984 fi gures (Hoglund, 1990) yet seed harvested in 1983 and 1984 had a range of hardseededness from 80-98% which should have ensured a higher survival. In addition, the numbers of soil-borne seeds averaged for all cultivars dropped steadily from 4200 m^2 in 1983/84 to only 1200 m^2 in 1987/88-losses not accountable for in terms of seed set or false strike. Such apparent losses of hard seed, possibly due to disease or degradation need investigation.

Oestrogenicity

Subterranean clover pastures can cause major problems with lambing and calving. Ingestión of phyto-oestrogenic substances by grazing subterranean clover leaf and to a lesser extent by ingesting hay, by breeding sheep or cattle over the weeks prior to mating causes temporary infertility which can reduce lambing and calving to as little as 8%. There is however no danger from naturally dried off herbage (Davies and Dudzinski, 1965). The symptoms are a general disturbance of reproductive function, uterine prolapse, and dystokia (Bennetts *et al,* 1946). Prolonged exposure causes permanent infertility following encystment of the uterine wall (Collins and Cox, 1985), and thinning of the mucous strands leading to inability of sperm to migrate through the cervix to achieve fertilisation of the ovum (Bennetts *et al,* 1946). The problem is caused by the isoflavones- formononetin, biochanin A, and genistein, of which formononetin is considered to be the principal causal compound (Millington *et al,* 1964a & b). The leve] of isoflavones in the herbage is increased by low soil fertility (Rossiter and Beck, 1966), and by leaf diseases (Jagusch, 1982).

The level of oestrogen in subterranean clovers is a varietal characteristic (Davis and Dudzinski, 1965). The early-flowering variety 'Dwalganup', widely used in the 300 to 500mm rainfall áreas of Australia since the earlyl930's, particularly in Western Australia, possesses high levéis of oestrogens. Although associated crop yields were good, animal problems were serious. 'Yarloop' and 'Dinninup' are also classified as having high levéis of isoflavones. 'Northam', 'Geraldton', 'Woogenellup' and 'Clare' have intermedíate levéis, while 'Daliak', 'Bacchus Marsh' and 'Mount Barker' have low levéis.

Testing for oestrogen level is an established priority in the selection process during the evaluation of new introductions of subterranean clovers (Francis *et al,* 1970). Those chosen for further evaluation must be below 0.2% DM formononetin in the leaf (Nichols, 1987).

On the basis of formononetin content, rather than total isoflavenes, lines tend to segregate on the basis of subspecies. In a large collection from Sardinia, Piano *et al.* (1993) found that compared to the non-oestrogenic cultivar 'Seaton Park', only 5% of lines from *T sub.* subsp. *brachycalicynum* var. *brachycalicynum* had a higher level. of formononetin, whereas 33% of lines from *Tsub.* subsp. *sub.* var. *subterraneum,* and 75% of lines from *T sub.* var. *yanninicum* did so.

Choice of variety

In 1950 a project to select more appropriate ecotypes of subterranean clover for the wheatbelt was started by Dr A.J. Millington in Western Australia. This evolved over the years to become the (Australian) National Subterranean Clover Improvement Programme (Collins, 1987). The Programme was administered and run jointly by the Western Australian Department of Agriculture and the University of Western Australia, with interstate collaboration from all states except Northern Territory. Associated was the establishment at Perth, of the Australian *Trifolium* Genetic Resource Centre, following a number of collecting expeditions to the Mediterranean región between 1951 and 1967 (Donald, 1970). The ongoing evaluation proceedure for subterranean clover involved the crossing of accessions and selection for up to eight years; screening for isoflavones, disease and pest tolerance, and then evaluation in each collaborating state. Then in 1997, after evolving through many intermediate bodies, the Programme became the National Annual Pasture Legume Improvement Programme, and was broadened to include other alternative legumes (Dear, pers. com.). The original programme and its successors have resulted in the propogation and reléase of cultivars with superior attributes for all the áreas of use of this species in Australia. The culmination of this work is the approval, naming and reléase of new cultivars. This is accomplished by listing with the CSIRO Australian Herbage Plant Registration Authority, and the publication, in the Australian Journal of Experimental Agriculture, of full details of parentage, screening results, productivity, special attributes and recommended áreas of use of the new cultivar. For instance Volume 32, 1992, pp 539-42. documents the reléase of one *T. sub.* var. *yanninicum* cv 'Gosse'; and three *T. sub.* subsp. *subterraneum* var. *subterraneum* cv 'Denmark'; cv 'Leura'; and cv 'Goulburn'. Full details of Australian pasture plant cultivar releases, including all subterranean clovers prior to 1990, can also be found in Oram (1990) and Barnard (1972). The information system is a model which could with benefít be emulated in other countries. It facilitates the publication for farmers of extensión publications such as" Subterranean clover in New South Wales-identifícation and use" by Dear and Sandral (1997). This gives details of a wide range of recommended subterranean clover cultivars, many of which are recent releases from the NAPLIP ; and all of which embody signifícant improvements in performance, disease and pest tolerance, as well as low levéis of antiquality agents. Table 2 summarises some of the information given in this publication.

TABLE 2
Characteristics of subterranean clover varieties recommended for use in New South Wales (after Dear and Sandral, 1997).

Características de las variedades recomendadas de trébol subterráneo para uso en Nueva Gales del Sur

Variety	**Flowering starts:**	**Days from sowing in mid-May to flowering d.**	**Mínimum rainfall for persistence mm**	**Hardseed in autumn 0 = nil 5 = high**
Nungarin	early Aug	110	375	**5**
Dalkeith	late Aug	120	400	**5**
Seaton Park L.	early Sept	125	475	**3**
York	early Sept	125	475	**5**
Trikkala	early Sept	122	525	**2**
Riverina	mid Sept	128	500	**3**
Rosedale	mid Sept	120	500	**3**
Gosse	late Sept	136	650	**3**
Junee	mid Sept	138	500	**3**
Woogenellup	mid Sept	140	525	**2**
Clare	late Sept	142	650	**2**
Goulburn	late Sept	145	525	**3**
Denmark	early Oct	149	600	**2**
Leura	early Oct	156	750	**1**
Nuba	early Oct	152	700	**3**

The choice of variety for a given área will hinge initially on the date at which moisture stress can be expected to occur and the time of flowering required to allow the maturation of adequate seed before this occurs. Theoretical considerations can be applied to arrive at an appropriate time of fírst flower appearance and as a result choose a variety for a given situation. Between 38 days ('Geraldton') and 54 days ('Dwalganup') were required from anthesis to allow máximum weight per seed to develop, both for buried and surface burrs (Tennant, 1965) although viable seed was formed earlier at 30 days and 38 days respectively. Consequently to set some seed and mature this, a variety needs to start flowering four or five weeks before soils fall below wilting point. However to set high yields of seed Dear and Sandral (1997) consider that flowering needs to continué for at least six weeks, with a further period of four weeks to allow full maturation of the seed formed- a total of 70 d. Rossiter (1978) suggests that a slightly longer period of about 80d from start of flowering is needed to maximise seed production.

Rossiter (1959) introduced the idea of describing the time of first flower appearance of varieties by using the number of days which had elapsed between the flowering of 'Dwalganup'; one of the earliest varieties, and the variety in question. He called this figure the maturity grading (MG). Thus 'Mount Barker' had an MG of 98 since this variety commenced flowering 98 days after Dwalganup. This system eliminates from varietal descriptions the fact that the time between germination and first flower appearance is shorter, and particularly so for later varieties, when sowings, or the natural time of germination, are progressively later (Dear and Loveland, 1984), and that this period differs between sowings made in western and eastern Australia (Dear and Sandral, 1997).

On average in Canterbury, New Zealand , in the southern hemisphere, soils reach wilting point by mid-November (Garnier, 1958) in early summer. Thus for a variety to be agriculturally successful from the seed production point of view, it needs to start flowering at least ten weeks before this date ie by 1 September in early spring. Observations by Smetham (1977) record that in 1969 'Dwalganup' flowered on 1 August (Maturity grading-MG 0); 'Geraldton' 14 August (MG 14); 'Yarloop' 3 September (MG 34); 'Woogenellup' 14 September (MG 45); 'Mount Barker' 5 October (MG 66) and 'Tallarook' 13 October (MG 74). Under similar conditions of rainfall and climate, Scott (1969) obtained adequate yields (499-739 kg ha^{1}) of seed from 'Geraldton' and 'Woogenellup', but cultivars flowering later than 'Woogenellup' set insufficient seed (70-239 kg ha') for success. On the other hand, at the high rainfall site with no moisture stress until late December, the highest seed yields (1100-1400 kg ha') were given by the late varieties 'Mt Barker' and 'Tallarook'.

Cultivars and numbered accessions from the Australian *Trifolium* Genetic Resource Centre, Perth, were also evaluated in Canterbury, New Zealand (Lat. 43 deg 50 min S) on shallow stony soils with an evenly spread rainfall of 650 mm/yr (Smetham *et al,* 1993). Out of 47 lines, only 22 produced more than 240kg.ha of seed. These flowered 99-138d after sowing at or before 18 September (Smetham *et al,* 1994), equivalent to a maturity grading of MG 9 to MG 48. They were in the early mid to mid season flowering group. Only one of the named cultivars tested 'Geraldton', was successful in terms of seed production. However in this región rainfall can be erratic, and an evaluation conducted during the next year (Smetham *et al,* 1994), when an atypical drought occurred in mid spring, showed that early and mid season flowering varieties were severely disadvantaged by this. Successful lines were all numbered accessions in the mid season and late mid- season flowering group, flowering up to 146 d after sowing, with MG 46-56. They all possessed an ability to recommence flowering after a check to growth-a characteristic termed "kick-on" by Australian agronomists. This is known to be possessed most strongly by annual medies, but also by subterranean clover lines with stout stems and peduncles (Gladstones, 1985).These results suggest that in áreas of variable erratic spring rainfall patterns, it is wise to include both early, and later flowering varieties of subterranean clover in the sown mixture to provide some insurance should one fail; a recommendation also made by both Gillespie and Nicholas, (1991) and Dearand Sandral (1997).

In Western Australia Rossiter (1966) examined the performance of 51 strains with MG from 0 ('Dwalganup') to 62 ('Wenigup'), and found that seed production was successful only where MG was less than 25, this again being a reflection of time of flowering relative to the early onset of water stress in this environment.

It will be evident that whilst seed production is a fírst criterion to be used in selection of a variety for a district, herbage production, hardseededness and other attributes including disease and pest tolerance must be taken into account. The results of Dear *et al*, (1993) show that while early varieties were successful in terms of seedset in their evaluation, the use of these early varieties was not justified in áreas of New South Wales with a rainfall of greater than 500 mm, since they were unable to take advantage of the extended season for growth.. Later varieties set sufficient seed too, but yielded around 10 t DM ha', while early varieties produced only 4 t DM ha'". Similarly Smetham and Jack, (1995) record that whilst from a seed production point of view early mid-season flowering varieties were superior, one mid-season, and two late mid-season flowering accessions were amongst the six highest herbage producers.

The recognition of characteristics needed in a cultivar for a given district, with regard to time of flowering relative to onset of drought, disease and pest tolerance, and level of hardseededness required , can form the basis for the definition of an ideotype. This can be a useful exercise prior to the selection of a variety for a given district, an example of which is given by Evans (1993); and by Dear *et al.* (1992), which resulted in the selection of cv 'Goulburn', a replacement for 'Woogenellup', but with the greatly improved disease resistence and a higher level of hardseededness as defíned in the ideotype.

Hardseededness is an attribute which might be thought to have greatest valué in áreas experiencing summer rainfall, however Evans (1993) found seedling losses at 25% were the same for both nominally "hard" or "soft"-seeded varieties. It is probable that hardseededness failed to develop at signifícant levéis in the cool températe conditions and evenly spread 500-700 mm annual rainfall at his location because of the lack of dehydration necessary for full development of this condition (Aitken, 1939). It would seem therefore that hardseededness will be both developed more fully and be of most valué in minimising losses to false strike in áreas with highly variable, unpredictable and sporadic summer rainfall rather than where this is regular and predictable.

An evaluation of nine subterranean clover cultivars at eight hill country sites in New Zealand (Williams *et al,* 1990) found that of overiding importance for success was the ability of a cultivar to survive and set seed under the cióse and continuous grazing; often to within 10-20mm of the ground surface, which is a feature of New Zealand hill country management (Sheath and Macfarlane, 1990b). This over-rode the importance of time of flowering, however all but two sites had rainfall of between 1200-1500 mm, with appreciable rain falling in summer. Such a climate meant therefore that most varieties, regardless of date of flowering, could survive and produce some seed. Overall 'Tallarook' was the most successful cultivar, being consistently high yielding with good regeneration (Chapman et al., 1986), and forming a model for the ideotype proposed for North Island hill country by Sheath and Macfarlane (1990a), of a variety flowering in late October-November, with prostrate crown and runner habit, and a low level of oestrogenic activity. Results show that the cultivars 'Woogenellup', "Clare, and 'Nangeela' (Sheath and Macfarlane, 1990a) and these together with 'Seaton Park' (Sheath and Macfarlane, 1990b) were unable to persist and regenérate under continuous cióse grazing. These cultivars have an open habit of growth, and the runners do not hug the ground as closely as the more persistent 'Mount Barker' and 'Tallarook', with the result that portions of runners with flowers and seed attached were often removed during grazing. The more persistent 'Larissa', 'Mt Barker' and 'Tallarook' also had smaller leaves contained in a dense growth habit and were very prostrate (Chapman and Williams, 1990).

Seed set initially ranged from 300-3000 seeds m^2 (Widdup and Turner, 1990; Macfarlane *et al,* 1990) and occasionally up to 7500 seeds m^2 (Sheath *et al,* 1990), but at most sites this declined over the years to low levéis, as a result of increasing competition from browntop *(Agrostis spp.)* and ryegrass *(Lolium spp)* (Sheath and Macfarlane, 1990a). It is unfortunate that at no sites was hardseed assessed. At most sites 'Tallarook' gave the best seedset and seedling numbers. However at only three of 63 site x cultivar records reported by Chapman *et al* (1986) did seedling numbers reach the 1000 m^2 suggested by several Australian authors eg. Taylor *et al.* (1984) as being a minimum for success. 'Woogenellup', 'Nangeela' and 'Howard' were frequently reported to lose considerable numbers of seedlings killed by desiccation (Sheath and Macfarlane, 1990b). Rains would have been experienced at most sites during seed maturation, and this moisture would have tended to minimise the full development of the hardseeded condition (Collins *et al,* 1966). It is significant that at two sites; Porangihau, with a rainfall of 700-900 mm , and Carvossa with 657 mm, the numbers of seeds set; 1800 to 3000 m^2 and 1200-4000 m^2, and seedling numbers of 330 m^2, and 500-700 m^2 respectively (Chapman *et al*, 1986), were maintained or even increased during the period of evaluation. This is not surprising since at both of these sites the climatic conditions were closer to those for which subterranean clover is adapted.

A more recent evaluation of North Island, New Zealand, hill country selections of subterranean clover; Australian breeding material; and some Australian cultivars (Dodd *et al,* 1995) again showed that 'Tallarook' was superior, although six other lines were similar, with the ability to consistently genérate winter populations of 200 plants m^2 in a mixed sward, and give more than 1000 kg DM ha' in spring.

Establishment

As already mentioned, the minimum desirable seedling population for puré swards after germination is at least 1000 seedlings m^2 (Taylor *et al,* 1991; Cocks, 1974). Machine harvesting of legume seeds normally substantially reduces hardseededness due to abrasión in the thrashing process. As a result germination is likely to be at least 75% and perhaps as high as 95%. With an average seedweight of 6g /l 000 and germination of 75%, the theoretical seed rate for a puré stand to acheive minimum seedling density in the year of sowing is therefore between 63 and 80 kg ha'. However in practise this is too expensive, although dairy farmers on the east coast of Australia sometimes sow around 20 kg ha'. The time taken by a puré stand of subterranean clover to achieve minimum seedling density for success from a low initial seedrate is however likely to be quite short because at low density plants set more seed per plant than at high density (Donald,1954). A sowing rate of 25 kg ha' produced 149-305 seedlings m^2, and set 247-345 kg ha^1 seed in the first season (Dear *et al,* 1993): quite adequate when compared with the suggested minimum for success discussed earlier. There is no record for the second season, but between 2000 and 6000 seedlings m^2 were counted in the third. In New Zealand, Calder (1951) found that it was not until the second season after sowing at 4.5 kg ha' that a dominant subterranean clover sward developed.

Puré stands in the eastern Australian wheatbelt have in the past been established using 1-2 kg.ha of seed sown under the last crop of wheat (Moore,1970), but recent extensión advice (Dear and Sandral, 1997) recommends 7 kg ha' on dryland and 10 kg ha' for irrigated country. In Western Australia the sowing rate after wheat has been low-often less than 2-3 kg ha' but in the 1960's recommendations have been to sow 9-13 kg ha' of seed (Rossiter and Ozanne, 1970)

Since the 1980's, problems have been experienced with establishment from undersowing in wheat, particularly in New South Wales. While sheep prove less profitable than wheat, farmers have been reluctant , in an attempt to maximise profitability, to reduce the seeding rate of the last wheat crop in the cropping phase of the rotation, increasingly undersown with subterranean clover. In addition clover seed sown directly behind the wheat coulters tends to be sown too deeply for good emergence. These conditions have led to a slower than usual build up in clover plant numbers (Dear, 1988). Alterations to equipment (Sykes, 1988), and a halving of the crop sowing rate or a doubling of clover sowing rate to 7 kg ha', have corrected this problem (Dear, 1988). In addition the period in wheat has been extended from two or three crops to four or five, which has exacerbated the problem , since the varieties of subterranean clover used ('Woogenellup' and 'Seaton Park') do not have particularly high levéis of hardseededness to allow them to persist through a series of crops without resowing. Selection of varieties for use in the wheatbelt has now placed more emphasis on hardseededness (Nichols, 1987). Higher rates are used on wetter country, and on dairying land in the southwest córner of Western Australia rates as high as 25 kg ha' have been used.

In New Zealand 2-4 kg ha' of subterranean clover has been used for oversowing onto hill country with an existing grass cover, or for sowing into cultivated ground together with a grass or grasses (Levy, 1970). Although establishment from oversowing into an existing sward increased continuously and significantly with sowing rate, Smetham (1980) found that an arbitrarily acceptable population for this purpose of 35 plants m^2 was obtained from sowing 9 kg ha' of seed. Subterranean clover has never expressed its full potential in New Zealand, probably because it has been sown at too low a seed rate, but also because it is rarely if ever sown as a puré sward (Smetham, *loe. cit.).*

Management to ensure re-establishment

Interpretation of Grime's theory of plant survival strategies in Grime *et al.* (1990) suggests that subterranean clover belongs to a group of plants classified as having a survival strategy almost "ruderal". Such plants can cope with considerable "disturbance" ie destruction of biomass by grazing or trampling, but only modérate "stress" ie., shortage of water, nutrients and only minimal competition for light. However subterranean clover avoids the stress of summer moisture déficit by surviving over this period as seed.

Stress typically causes increased reproductive activity in ruderal plants viz. the increased seed production of subterranean clover following defoliation (Collins, 1981); and increased burial of burrs (Collins *et al,* 1983), neither of which increase the ability to compete, although survival is enhanced. Tolerance of competition is low, although this is assisted in legumes since they fix their own N. Competition from associated grasses is made more acute by the increase of soil N associated with continued clover growth

(Helyar *et al,* 1997), however grazing "disturbance" which subterranean clover can tolérate, can minimise this. Where the stress is less extreme ie where there is significant effective rainfall during the dry period, perennial grasses can and do pose a competitive threat to subterranean clover.

Severe competition from perennial grasses and lúceme has a major effect on seedlings (Dear and Cocks, 1997) with survival at the end of March of 57% when subterranean clover seedlings were growing with phalaris (summer inactive), 13% with a danthonia, and only 1% with cocksfoot (both summer active). By mid May, only 2 seedlings m^2 survived in any of the perennial companion plant plots, compared with 964 seedlings m^2 in the puré subterranean clover. A decline in the moisture content of the surface soil horizons indicated that competition for moisture was involved. Subsequent work (Dear *et al,* 1998) indicated that competition for light and nitrate as well as water, is involved when perennials are growing with establishing subterranean clover seedlings. Defoliation of the perennial grass can reduce competition both for light and water to the benefít of the clover seedling numbers.

It must therefore be appreciated that it is vital for the agricultural success of this plant that competition is minimised during natural seedling re-establishment to ensure that an adequate population survives. In practise this can be achieved by thinning out one third of the perennial grass tillers using light cultivation with grubber, discs or heavy harrows, followed by rolling, in midsummer every two to three years (Calder, 1954), or by very heavy grazing in the weeks prior to the commencement of germination .

Future productivity is assisted by spelling the germinating stand from grazing for some three to fíve weeks, so allowing leaf área to build up (Silsbury and Fukai, 1977). This helps the clover compete with other vegetation, and also enhances production due to the compounding effect of leaf área on cool season growth (Hoglund and Pennell, 1989).

Each year of subterranean clover growth increases soil N (Watson and Lapins, 1964; Dear *et al,* 1999) and therefore associated grass competitiveness. This can be minimised by two or three years of cropping to reduce N levéis (Watson and Lapins, 1964). However even if soil N levéis are only modérate but grazing is lax and infrequent, then seed production, and the re-establishment of subterranean clover is likely to be low (Dear *et al,* 1998, 2000) and it will eventually disappear.

This problem is particularly hard for hill country farmers to solve. Typically farmers on such country (Emmersen, 1980) obtain the necessary degree of topgrowth control by rotating hard, clean-up grazing around a small number of paddocks in summer and autumn, in any one year, so that cover removal is achieved at least one year in three. It is possible to use a selective grass-killing herbicide to achieve control, and Smetham (1980) obtained a sixfold increase of seedlings establishing in a herbage mass of 3100 kg DM ha' after spraying. Subsequent seed production showed a four fold advantage over no spraying.

Use as **a puré sward or in mixture with a** grass

The question of whether subterranean clover confers most benefit as a puré sward or mixed with a grass-either annual or perennial, needs to take into account many considerations including herbage yield, interactions between quality and yield, timing of herbage production, and stock requirements in terms of quality and yield. Puré swards are seen in Autralia as responsible for soil acidifícation and do little to stem rising water tables, and are rapidly invaded by weeds.

In most of températe Western Australia, and the drier parts of the wheatbelt in eastern Australia the summer climate is too severe for perennials to survive (Moore, 1970b), but elsewhere in Australia subterranean clover is used in mixture with perennial grasses where the climate allows these to survive. In eastern Australia where 35-60% of rain falls in summer (Gentilli, 1971b), grasses in mixture with subterranean clover are used, depending on total rainfall (Moore, 1970b). *Phalaris,* and more recent selections of *Dactylis* can be used where this is 450-630 mm per year. However the survival of some of these dryland adapted cultivars eg *Phalaris aquatica* cv 'Sirocco' is dependant on a degree of summer dormancy, henee productivity may not be significant over this period (Cooper and Tainton, 1968) Below 450 mm in Victoria and South Australia, the annual ryegrass *Lolium rigidum* Gaud. is used in combination with subterranean clover (Donald, 1970). Above 630 mm perennial ryegrasses can survive for use in mixed pastures (Moore, 1970b).

In New Zealand subterranean clover is almost invariably used in pasture mixtures, which include perennial ryegrass *(Lolium* spp.) and sometimes cocksfoot *(Dactylis* spp.), The contribution of subterranean clover to the yield of these mixed pastures in New Zealand; up to 1500 kg DM ha' in hill pasture (Ledgard *et ai,* 1987) or 1300 kg DM ha[1] in fíat dryland pasture on stony soils (Rickard and Radcliffe, 1976), is not particularly impressive, whereas experimental sowings of puré subterranean clover have given substantial yields of 8000-13000 kg DM ha[1] (Suckling, 1960; Scott, 1971). In addition a puré sward of subterranean clover will give cool-season growth rates as high as any perennial grass (Smetham and Jack, 1995).

However there will be little production of green herbage from mid-summer until rains in autumn. Whilst stock can exist over summer at a maintainance feeding level on dried off herbage and burr at stocking rates of up to 6 dry sheep equivalents/ha (de Koning and Cárter, 1989), provisión of crop or greenfeeds is needed to allow flushing of ewes before joining with the ram. The inclusión of dryland persistent perennial grasses with summer production capability in mixture with subterranean clover in New Zealand, eg. 'Grasslands Wana' cocksfoot (Barker *et ai,* 1985), or 'Grasslands Maru' (Stevens *et al,* 1989), or as already noted as being practiced in Australia (Moore, 1970b), will provide grazing in summer, but overall herbage quality will be lower.

Although the digestibility of grasses and clovers under grazing may be similar, the greater intake of clover herbage leads to higher growth rates of stock (Ulyatt *et al,* 1977). Consequently animal growth rates are likely to be greater on puré, than on mixed grass-clover swards.

There are particular problems in Australia concerning the acidification associated with legume nitrogen fíxation (Ridley *et ai,* 1990), and with rising water tables and salinisation (Badén Williams, 1991). Both these problems are accentuated by the widespread use of puré swards of shallow-rooted subterranean clover. It is now widely recognised (Dear, pers. com) that by growing deeper rooting perennials, including lúceme and trees, with subterranean clover can minimise or prevent acidification (Porter, 1981) and salinisation (Schofíeld, 1989) by minimising the downward movement of water through the soil profile.

Although subterranean clover may be sown as a puré sward, in practice this is rapidly invaded by volunteer annual broadleaved weeds like capeweed *(Arctotheca* spp.), and annual grasses such as *Vulpia* spp. *Lolium* spp. and *Bromas* spp., and such stands are impossible to maintain without the extensive use of herbicides (B.S. Dear, pers. com.). However this is especially so when absence of competition resulting from a low subterranean clover population occurs.

With mixed grass and subterranean clover pastures the insulating effect of continuing herbage cover over the summer period (Quinliven and Millington, 1962) plus lower summer temperatures eg. Armidale with a January mean extreme day temperature of 40 deg C compared to Geraldton, Western Australia, of 48 deg C (Gentilli, 1971b) can cause hardseededness to be lost more slowly (Fitzgerald, 1987), resulting in clover germination levéis often marginal for success.

Allellopathy is a problem associated with the use of grass with subterranean clover. Residues from *Phalaris* have been shown to significantly reduce germination and aspects of root growth in subterranean clover seedlings, with many of the Australian cultivars being severely affected compared to lines from grassy environments in the Mediterranean (Leigh *et ai,* 1995). Breeding strategies to capture resistance are predicted to lead to an improvement in new releases.

In the previous section it was argued that subterranean clover has a ruderal survival strategy, and as such is not a strong competitor (Grime *et al*, 1990). In the Mediterranean native habitat of subterranean clover, competition from perennials is unlikely since the absence of rain is almost complete for months, and competition from other annuals is not weighted as heavily in favour of the competitor as it is with a perennial having a permanent root system and crown. However where climate allows perennial grasses to survive, the use of these with subterranean clover is likely to severely restrict its ability to perform to potential. In the absence of special circumstances which demand the use of a grass with subterranean clover, the strategy with greatest benefit to a pastoral system may on balance be to use subterranean clover as a puré stand.

Herbage production

Differences in flowenng time has implications for herbage production since leaf production slows dramatically once flowenng starts, and means that late flowenng varieties produce more herbage dry matter than those flowering earlier (Rossiter, 1959) although this can only occur if the moisture regime allows potential growth to be realised. Henee in an environment which is moist enough to support late season flowering varieties, herbage production is directly related to time of fírst flower appearance. Evans (1993) for instance found that yield increased by 43 kg DM ha' for every day later flowering occurred. In eastern Australia and with a long term average 530 mm annual rainfall, but extended season, two early cultivars 'Nungarin' (110 d from germination to lst flower at Wagga Wagga, New South Wales) and 'Dalkeith' (120 d) gave 4.1 t DM ha' and 4.6 t DM ha' respectively, whereas the later varieties 'Seaton Park' (125 d) and 'Mount Barker' (156 d) gave considerably higher yields of 11.5 t DM ha' and 10.5 t DM ha^{1} (Dear *et al*, 1993, Dear and Sandral, 1997). In this situation early varieties were clearly unable to take advantage of the extended season. Scott (1972) found the same in New Zealand with a progressive increase in yield from the earliest to the latest flowering varieties at the site where rainfall allowed this to occur.

Herbage yield is also affected by seedling population (Taylor *et al*, 1991), and by the date of germination in the autumn (Silsbury and Fukai, 1977). Early autumn applications of N to pasture, have been shown to increase leaf área, leading to increased winter and spring DM yields (Hoglund and Pennell, 1989). Similarly the early germination of subterranean clover results in leaf área which builds up early and quickly, before the cooler and shorter days of early winter, and this leads to higher winter growth rates than where sowing or germination is later (Blumenthal *et al*, 1993). Swards sown in mid-

April or late May give little winter production compared to those sown in early March (Dear and Loveland, 1984). Silsbury and Fukai (1977) found that total end of season yields were also dependant on time of germination but while winter growth was maximised by early germination, total yield was highest from a May sowing. In New Zealand total production of subterranean clover-grass pasture is closely related to spring rainfall and spring herbage growth, which generally constitutes two thirds of total yield (Iversen, 1957)

In Australia total DM yields exhibit a huge range. Cocks (1974) has recorded a máximum total yield of 16.4 t DM ha', finding yield strongly dependant on plant density and leaf área, but Silsbury and Fukai, (1978) measured growth rate increasing with leaf área index up to a herbage mass of 2000 kg ha' but decreasing beyond 6000 kg ha' as respiratory load increased.. Calculated yields (Cocks, 1974) rose from 7.7 t DM ha' with a sustained LAI of 2, to 13.2 t DM ha' where LAI was maintained at LAI 5.

The potential for yield is strongly influenced by the length of the growing season (Silsbury and Fukai 1978), which necessarily equates with autumn, winter and spring - early summer rainfall. Blumenthal *et al* (1993) recorded an average over three seasons of 6000 kg DM ha'. at Forbes with rainfall of 280-301 mm and additional water to prolong the growth. These authors obtained a very strong correlation (r=0.99) between water use and DM yield. Increased use of water gave increases of production up to 11 t DM ha' from 600 mm water used. At Wagga Wagga with a long term average 530 mm rainfall, Dear and Loveland (1984) obtained yields up to 10.3 t DM ha' from a mid-season variety.

Productivity of subterranean clover in áreas other than the wheatbelts where it may be grown with an annual grass like Barley grass *{Hordeum murinum)* or more commonly with a perennial like *phalaris* or perennial ryegrass does not seem to be well documented. In áreas of Australia with soils of pH 5.2-7.5, having good drainage, and a rainfall of more than 350-400 mm. year, lúceme can be a productive pasture option (McDonald and Waterhouse, 1989). However little herbage is produced over winter. The sowing of subterranean clover with the lúceme can correct this imbalance (Fitzgerald, 1979; Wolfe and Sutherland,1980). Where suitable conditions occur a proportion of the wheatbelt in both eastem and western Australia is sown to this mixture, the two species of which give additive and complimentary production (Wolfe and Sutherland, 1980) with good persistence as long as the lúceme is sown at a wide row spacing.

Almost without exception the mid-season flowering 'Mount Barker', and the late season flowering 'Tallarook' have been the varieties extant in New Zealand hill country (Suckling *et al,* 1983) and on fíat to rolling terrain (Saxby, 1956). On steep North Island

(NZ) hill country Ledgard *et al*, (1987) have measured total sward productivity of 5-6400 kg DM.ha' with subterranean clover contributing 1200-1500 kg DM ha'.

In varietal triáis on hill country 'Mount Barker' and particularly 'Tallarook' were generally the most productive varieties (Sheath *et al*, 1990; Chapman and Williams, 1990) giving totals of more than 2000 kg DM ha^{1}, with 'Seaton Park', 'Trikkala', 'Howard' and 'Woogenellup' producing around 1500 kg DM ha^{1} initially (Sheath and Macfarlane, 1990a). However three seasons later these totals had decreased by 60-85% (Sheath and Macfarlane, 1990a) or more (Widdup and Turner, 1990) as a result of increasing competition from browntop *(Agrostis* spp.) and ryegrass *(Lolium* spp) (Sheath and Macfarlane, 1990a) and white clover (Widdup and Turner, 1990) and poorer regeneration. Use of 'Mount Barker' and 'Tallarook' on stony free draining soils of the Canterbury Plains in New Zealand has resulted in long term average total sward yields of 6000 kg.DM.ha^{1}, with subterranean clover contributing 1300 kg (23%) of this (Rickard and Radcliffe, 1976).

While this legume is very seldom used in New Zealand as a puré sward, various authors have shown puré swards to be highly productive, and possibly more so than where associated with a grass or grasses. Two years of results under periodic cutting and grazing (Harris *et al*, 1973) gave annual yields from 1000 kg DM ha' for early flowering varieties to 4400 kg DM ha' for mid and late season types, however there are no New Zealand comparisons of a puré sward with a mixed grass clover sward. Under a climate and on soils almost identical to those used by Rickard and Radcliffe (1976), Smetham and Jack (1995) obtained production from a large number of lines sown as puré swards, and rotationally grazed by sheep, the best three of which exceeded total production of 5000 kg.DM.ha under hard periodic grazing. Scott (1971) harvested 7900-8900 kg DM ha' from two early mid-season cultivars cut three times in a 550mm rainfall área of the South Island, New Zealand, while Suckling (1960) recorded 11000-13000 kg DM ha' from late cultivars cut monthly in a 1500 mm rainfall área of the North Island, NZ. These figures indicate that puré swards of this legume are capable of substantial productivity; often greater than that from the mixed grass clover swards above.

In a comparison with lúceme, Smetham and Jack, (1995) showed that a two year oíd stand of lúceme cv.'Grasslands Otaio' produced only 3310 kg DM ha^{1} compared an average of 5100 kg DM ha^{1} for the three best lines out of 18. Moisture in this season favoured subterranean clover during the cool season, whilst a very dry late spring-early summer disdvantaged lúceme productivity. However Vartha and Fraser (1978) quotes a four year average for dryland lúceme of only 8500 kg DM ha'. On the basis of these figures subterranean clover could be a more productive option used as a puré sward, and in total production may sometimes give similar production to lúceme in summer-dry áreas.

The use of broadleaf herbicides frequently used in south eastern Australia to control weeds in pasture has been shown to reduce the yield of all subterranean clovers, even when the chemicals are used at recommended rates (Dear *et al,* 1995). However considerable variation in reaction exists amongst cultivars with herbage yield being reduced 16-71 % depending on variety.

Herbage quality

The mature dry herbage of subterranean clover can be quite adequate as a maintainance feed for sheep, having a digestability of DM of 35-60% depending on variety and exposure to rain (Rossiter *et al,* 1994), and the dried off herbage and associated burr can feed up to 6 dry sheep equivalents.ha (de Koning and Cárter, 1989) depending on the previous green herbage productivity of the stand. Summer rain decreased dry matter digestability by 5-16 percentage points. Unlike most legumes, the digestability of leaf is less than that of stem and peduncle (Rossiter *et al,* 1994, Stockdale, 1992) due to a higher ratio of lignin to cell wall material in the leaf (Mullholland *et al,* 1996). In a grazing experiment it was not until the second year that signifícant differences in digestibility and liveweight gain between cultivars developed. However there are also differences in the energy required to shear the dried off herbage which are related to voluntary intake (Baker *et al,* 1993) and while this is to some extent dependant on the ratios of stem and petiole to leaf, there are marked varietal differences too. Stockdale (1992) reports that irrigated puré subterranean clover is an excellent feed for dairy cows, which produced up to 28 kg milk/cow in early lactation although he produced no comparative data for other legumes.

SUSCEPTABILITY TO PESTS AND DISEASE

Viruses

Viruses affecting subterranean clover have been reviewed by Johnstone and Barbetti (1987) and only brief comment about them will be made here. In summarising the situation, Johnstone (1987) made the following points. Virus diseases considered important at one time or another in Australia have been alfalfa mosaic virus, bean yellow mosaic virus, clover yellow vein virus, cucumber mosaic virus, soybean dwarf virus, (also known as subterranean clover red leaf virus),subterranean mottle virus and subterranean clover stunt virus. Since the mid-1950's when virus suddenly emerged as a problem in Australia, the relative prevalence of the various viruses has continually changed. Occurrence of virus epídemics has been sporadic and is generally localised in importance. Nevertheless losses of herbage can be 50% and more.

Two main classes of virus can be recognised. **The** first group are persistent viruses spread by aphid vectors from host plant species. These include subterranean clover stunt virus and subterranean clover red leaf virus. All 1987 registered subterranean clover varieties were susceptible. The second group of viruses are spread in a non-persistent manner, and are mostly transmitted through the seed of at least some of their hosts. These are alfalfa mosaic virus, cucumber mosaic virus, bean yellow mosaic virus and bean yellow mosaic virus. These have increased in incidence since the arrival in Australia **in the late** 1970's of the pea aphid *Acyrthosiphon pisum.*

Losses from the two most important virus diseases of subterranean clover in Australia: cucumber mosaic and bean yellow mosaic viruses, spread by aphid vectors, depend very much on the survival of infected seedlings and the rate of spread of infection, and were found to differ greatly from season to season (Jones, 1993). Herbage yield reduction ranged from 7-49% while seed yields were reduced by 14-64%. There is wide variation in subterranean clovers in reaction to the viruses; the vectors, and to transmission via seed, and there is evidence that good tolerance exists in some lines held in the subterranean clover section of the *Trifolium* Gene Bank operated by the Department of Agriculture, Western Australia, Perth, which will allow resistant lines to be selected.

In New Zealand Ashby (1980) has surveyed viruses of annual legumes, and notes stunting of subterranean clover from bean yellow mosaic virus; a wide incidence of alfalfa mosaic virus with blue green aphid as vector; and the prevalence of subterranean clover red leaf virus and aphid vector *Aulacorthum solani* (syn. *Acyrthosiphon solaní)* (Ferro, 1978) overwintering on white clover, to cause infection of subterranean clover **in** spring.

Foliar fungal diseases

In Australia the most prevalent and damaging fungous disease is Clover Scorch *(Kabatiella caulivora),* which causes necrosis mainly at the junction of petiole and **lamina** followed by collapse of the leaf and the browning off of the whole canopy. The disease is worst, and spread most rapid, in puré swards closed for seed or hay, and growing on heavy soils. It can be so devastating that screening of introductions and crossbreds for susceptibility or otherwise was a priority in the Australasian Subterranean Clover Improvement Programme. There are wide differences in tolerance or susceptability of diseases amongst varieties, which allows selection of resistant lines

(Barbetti and Gillespie, 1987).This disease occurs on red clover hay crops in New Zealand at wet sites, but is not recorded on subterranean clover (Cióse, 1990), probably because this species is almost always grown on free-draining soils, and seldom if ever saved as a puré sward for hay.

Rust disease *(Uromyces trifolii)* is sporadic and can be severe locally in Australia, and has been found on New Zealand stands of subterranean clover (Cióse, 1990). Screening for this problem was routinely carried out in subterranean clover improvement programmes (Barbetti and Gillespie, 1987).

These authors also list a number of other foliar diseases, including *Cercospora zebrina* which can be serious. In a review of pasture diseases occurring in New Zealand, Cióse (1990) likewise mentions a number of fungous diseases affecting clovers but few were noted on subterranean clover with the exception of *Cercospora zebrina,* which causes reddish leaf spotting. Cióse (1990) cautions that many of the leaf spotting diseases cause the host to produce oestrogenic substances potentially detrimental to animáis.

Root fungal diseases

Several organisms can be responsible for root rots in subterranean clover including *Pythium, Fusarium, Rhizoctonia, Aphanomyces,* and *Phytophthora clandestina.* However it is mostly the latter that is involved in serious field infections (Greenhalgh and Flett, 1987). Losses can range from 30-70% and are worst when moisture stress with warm temperatures follows an early break or irrigation. The disease is sufficiently severe in some seasons in Australia for screening for resistance to this organism to be another essential preliminary in the selection of new varieties of subterranean clover (Flett *et al,* 1993). Lines have shown great variation in susceptibilty, with 22 % of the 800 tested up until 1988 being resistant. More than 95% of *T sub.* var. *yanninicum* lines tested were resistant, with the exception of 'Yarloop'. Whilst 'Larisa' is highly resistant, the older cultivars 'Woogenellup', 'Mt Barker', 'Northam' and 'Tallarook' are highly susceptible. In New Zealand root rots do not seem to cause problems with subterranean clover, although the crown rot *Sclerotinia trifoliorum* has been recorded on this species (Cióse, 1990).

Insects

Commenting on insect pests of subterranean clover pastures in Western Australia, Sandow and Gillespie, (1987) consider that although there are a variety of moth larvae, beetles, weevils , and springtails which do attack clover, the most damaging pests are the red legged-earth mite *(Halotydeus destructor),* and the blue-green aphid *(Acyrthosiphon kondoi).* The red-legged earth mite causes mechanical damage resulting from feeding lesions on established plants but more important is the very high mortality of seedlings which can result if attack occurs shortly after germination. This pest is only of concern in áreas with winter rainfall and an absence of summer rain (Wallace and Mahon, 1971). In áreas of summer rain, the very similar blue oat mite is of equal importance in causing damage (Dear and Sandral, 1997). Screening for resistence to red-legged earth mite, which is widespread amongst lines, has been conducted as part of subterranean clover breeding in Australia since 1985.

The blue-green aphid debilitates plants by feeding activity which, if aphid populations are high enough, can cause plants to die off prematurely. However more serious is the transmission of virus diseases which can occur, even with the low population of aphids which frequently overwinter on clover stands. To date screening triáis have not been conclusive. Since blue-green aphids also arrived in New Zealand in the late 1970's, it is likely that they may be involved in virus spread and reduction of plant vigour in that country also (Ferro, 1978).

Pasture cockchafer *(Aphodius tasmaniae)*, known as Tasmanian grassgrub in New Zealand, can in some seasons cause localised sporadic damage to subterranean clover in winter (Chapman, 1990), although in New Zealand it appears to be restricted to the driest shallow, stony, and sandy soils. Both adult beetles and larvae feed on the aerial parts of clover plants but larvae are more damaging since they feed until pupation in July. (Ferro 1978).

Whitefringed weevil *(Graphognathus leucoloma)* larvae, feeding on roots and nodules are reponed as causing localised and sporadic damage in New Zealand (Chapman, 1990), while the lúceme flea *(Sminthurus viridis)* is classified as localised and persistent.

FUTURE DIRECTIONS FOR SUBTERRANEAN CLOVER RESEARCH

Australia.

At the present time it is unlikely that further breeding and selection of subterranean clover and annual medies will proceed, although there may be releases in the future of material currently under evaluation. Under the present (Australian) National Annual Pasture Legume Improvement Programme emphasis has now shifted almost completely towards other annual legume species, including *Trifolium michelianum, T. glanduliferum, T. nigrescens, Ornithopus sativus, O. compressus, Trigonella balansae* and *Biserrula pelecinus* (B.S. Dear, pers. com.).

New Zealand.

By using climate, parent material and topography to assess the suitability of soil types for subterranean clover, áreas have been derived from the Soil Bureau Bulletin 26 of New Zealand soils (New Zealand Soil Bureau, 1968a). The total área of soils where subterranean clover is considered to have the potential to make a more significant contnbution to herbage production than white clover is 1.9 M ha (Smetham, loe. cit) out of a total of 14 M ha of soils under grassland. However Maunder (1971) maps a larger área of around 2.7 M ha of New Zealand as having a "slight to considerable defícieney of water" , which may indicate a place for this legume. Subterranean clover is currently used on only a fraction of the grassed área in New Zealand. Extensive áreas of shallow and stony soils particularly in Central Otago and the Mackenzie Country of Canterbury are too shallow to be economic to irrígate (New Zealand Soil Bureau, 1968b), and currently support an ephemeral vegetation dominated by the annual legumes such as Striated clover *{Trifolium striatum* L.), Clustered clover *(Trifolium glomeratum* L.), and Haresfoot Trefoil *(Trifolium arvense* L.), with an annual production of less than 1000 kg DM ha^{1} (Smetham and Jack, 1995). Subterranean clover has never been properly evaluated on such soils. Any evaluation programme would need to screen the large numbers of Unes already introduced from overseas by Australia, rather than assume that cultivars currently recommended for Australian regions could be suitable for superficially similar rainfall áreas in New Zealand. Such is unlikely, due to the influence of the many subtle parameters of climate and soils. In addition lines need to be assessed for total seed production and hardseededness in áreas where they will be used, rather than at a central testing área.

Longer term solutions to high proportions of hardseed in autumn in New Zealand are either to select lines with a faster rate of hardseed breakdown under a lower amplitude diurnal temperature regime; considerable variation for which has been shown to exist (Smith *et al,* 1996), or to use genetic manipulation to achieve the same result.

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ECOLOGÍA Y USO DEL TRÉBOL SUBTERRÁNEO *{TRIFOLIUM SUBTERRANEUM* L.) COMO LEGUMINOSA PRATENSE. UNA PERSPECTIVA AUTRALASIÁTICA

RESUMEN

Se revisa la investigación realizada en Australia y Nueva Zelanda sobre la ecología y el uso del trébol subterráneo *(Trifolium suhterraneum* L.), leguminosa pratense anual. Se describe la especie y su distribución, y los niveles de acidez y fertilidad de los suelos a los que se adapta. Se revisan brevemente las respuestas vegetativas a la luz y a la temperatura. El control mediambiental de la reproducción se describe en relación con la vernalización, el letargo de las semillas, el desarrollo y disminución de la dureza seminal, y el enterramiento de los glomérulos.

Se detalla la historia del uso del trébol subterráneo en agricultura. Se discute la importancia de la producción de semilla en el éxito agrícola de la especie, y el efecto del pastoreo en dicha producción. Los problemas asociados con la dureza seminal se relacionan junto con sus posibles soluciones. Se debate sobre la utilidad de la dureza seminal. Se detallan los síntomas asociados a la presencia de sustancias estrogénicas. Se ilustra el modo de elección de una variedad para una localidad determinada. Se describe el manejo para asegurar un buen establecimiento inicial y un buen re-establecimiento en los sucesivos otoños. Se debate sobre el uso de la especie como monofíta o en mezcla con gramíneas. Se relaciona la producción herbácea en varias localidades, y se discute sobre su calidad para la producción animal. Se relacionan las plagas y enfermedades, junto con una valoración de su importancia relativa. Se sugieren nuevos temas de investigación sobre trébol subterráneo.

Palabras clave: Manejo, reservas de semilla, dureza seminal.